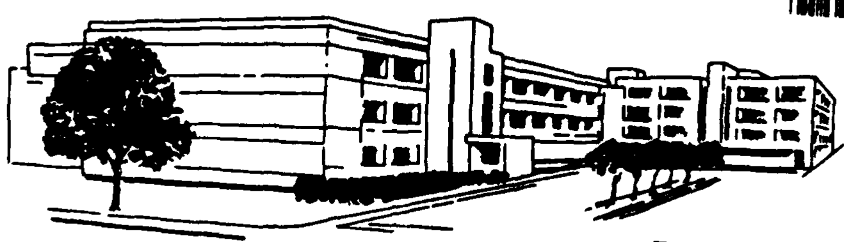
LABORATORY NOTE NO. 81

The Summer Apprentice Program
1992

Katherine Ellen Renn
Thornton Samuel Mu
David M. Dahle
Vincent K. Lee
Renee M. Ward

In Cooperation with George Washington University
School of Engineering and Applied Science Summer Apprentice Program

Summer 1992



LETTERMAN ARMY INSTITUTE OF RESEARCH PRESIDIO OF SAN FRANCISCO CALIFORNIA 94129

This material has been reviewed by the Commanding Officer, Letterman Army Institute of Research and there is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

Barbara A. Wilson, MAJ, MS, DCA
14 June 93

LABORATORY NOTE NO. 81

DTIC QUALITY INSPECTED 5

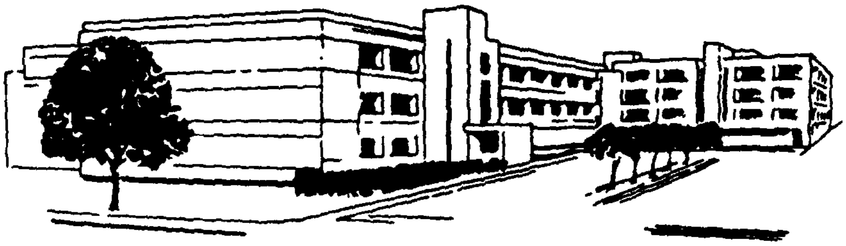
Accesion For	
NTIS CRA&I	☑
DTIC TAB	☐
Unannounced	☐
Justification	
By	
Distribution /	
Availability Codes	
Dist	Avail and / or Special
A-1	

The Summer Apprentice Program 1992

Katherine Ellen Renn
Thornton Samuel Mu
David M. Dahle
Vincent K. Lee
Renee M. Ward

In Cooperation with George Washington University
School of Engineering and Applied Science Summer Apprentice Program

Summer 1992



LETTERMAN ARMY INSTITUTE OF RESEARCH PRESIDIO OF SAN FRANCISCO CALIFORNIA 94129

DEPARTMENT OF THE ARMY
LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

REPLY TO
ATTENTION OF:

THE SUMMER APPRENTICE PROGRAM - 1992 -- KE Renn, TS Mu, DM Dahle, VK Lee, RM Ward

This document has been approved for public release and sale; its distribution is unlimited.

Destroy this report when it is no longer needed. Do not return to the originator.

Citation of trade names in this report does not constitute an official endorsement or approval of the use of such items.

This material has been reviewed by Letterman Army Institute of Research and there is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official nor as reflecting the views of the Department of the Army or the Department of Defense. (*AR 360-5*)

for Barbara A Wilson, MAJ, MS, DCA

John R. Hess (date) 14 June 93
COL, MC
Commander

UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release; Distribution is UNLIMITED.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S): Laboratory Note # 81	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: Letterman Army Institute of Research; 6b. OFFICE SYMBOL (If applicable): SGRD-ULZ	7a. NAME OF MONITORING ORGANIZATION: US Army Medical Research and Development Command
6c. ADDRESS (City, State, and ZIP Code): Letterman Army Institute of Research, Bldg 1110, Presidio of San Francisco, CA 94129-6800	7b. ADDRESS (City, State, and ZIP Code): Ft. Detrick, Frederick, MD 21701-5012
8a. NAME OF FUNDING/SPONSORING ORGANIZATION; 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code)	10. SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. / PROJECT NO. / TASK NO. / WORK UNIT ACCESSION NO.

11. TITLE (Include Security Classification)
(U) The Summer Apprentice Program - 1992

12. PERSONAL AUTHOR(S)
KE Renn, TS Mu, DM Dahle, VK Lee, RM Ward

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final	FROM ____ TO ____	1992 Summer	

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	nitric oxide, pyridoxine, Hb oxygen binding, hypertension, post-thaw blood preservation

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

During the summer of 1992, Letterman Army Institute of Research hosted five inquisitive and energetic teenagers as part of the George Washington University School of Engineering and Applied Science Summer Apprentice Program. Each participant was assigned to a LAIR researcher who served as mentor. The pace was vigorous, and the work was challenging. On August 6, 1992, the students presented their final reports to the Commander of LAIR and other dignitaries from the Presidio of San Francisco. A few days later they flew to Washington, D.C. and presented their projects to the other program participants.

The final reports prepared by the summer apprentices are presented in this LAIR Laboratory Note.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT: ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION: Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL: John R. Hess, COL, MC, Commanding	22b. TELEPHONE (Include Area Code): (415) 561-2891; 22c. OFFICE SYMBOL: SGRD-ULZ

DD Form 1473, JUN 86 — Previous editions are obsolete. — SECURITY CLASSIFICATION OF THIS PAGE

Unclassified

Summer Apprentice Program

1992

During the summer of 1992, Letterman Army Institute of Research hosted five inquisitive and energetic teenagers as part of the George Washington University School of Engineering and Applied Science Summer Apprentice Program. Each participant was assigned to a LAIR researcher who served as mentor. The pace was vigorous, and the work was challenging. On August 6, 1992, the students presented their final reports to the Commander of LAIR and other dignitaries from the Presidio of San Francisco. A few days later they flew to Washington, D.C. and presented their projects to the other program participants.

The final reports prepared by the summer apprentices are presented in this LAIR Laboratory Note.

Katherine Ellen Renn

Bloomingdale Senior High School

Randolph-Macon Women's College

August 5, 1992

Letterman Army Institute of Research

Mentor - Raymond Regan, M.D.

The Effect of Nitric Oxide on NMDA Neurotoxicity

ABSTRACT

During N-methyl-D-asparate (NMDA) neurotoxicity, NMDA receptors, in response to glutamate, open their channels allowing an influx of intracellular calcium. The processes leading to cell death after an influx of calcium are unknown, but experimental evidence suggests it may be due to nitric oxide (NO). We showed, in our system, that the nitric oxide synthase inhibitors, N^{ω}-nitro-L-arginine and N^{ω}-monomethyl-L-arginine, and NO binding hemoglobin do not prevent NMDA neurotoxicity. These agents also blocked the formation of cyclic guanosine monophosphate. These data suggest that NO does not mediate the neurotoxicity of NMDA.

The effect of nitric oxide on NMDA neurotoxicity

Katherine E. Renn

Stroke is the third leading cause of death in the United States, claiming approximately 500,000 victims each year (1). Thirty percent of stroke victims die and 20% to 30% become permanently and severely disabled (1). Experimental evidence has supported the hypothesis that during stroke, excessive amounts of glutamate are released by ischemic neurons. Studies have suggested that in response to glutamate, N-methyl-D-asparate (NMDA) and DL-α-amino-3-hydroxy-5-methylisoxa-zole-propionic acid hydrobromide salt/kainate (AMPA/kainate), types of glutamate receptors, open their channels allowing the influx of calcium and sodium (1). AMPA/kainate receptors only allow the passage of sodium. An abnormal build-up of both ions may occur. The increased build-up of calcium may trigger the release of glutamate, thus spreading the calcium cascade to other cells (1). The excess calcium may activate proteases, lipases, and endonucleases. These enzymes may degrade nucleic acids, proteins, and lipids. The metabolism of arachidonic acid, a by-product of the degradation of phospholipids, leads to the formation of oxygen-free radicals (1). An overabundance of calcium also stimulates the formation of nitric oxide (2).

The mechanism for the formation of nitric oxide in the central nervous system (CNS) is still not fully understood. It is theorized that glutamate released from presynaptic terminals binds to NMDA

receptors. Calcium rushes in and binds with calmodulin, thereby activating NO synthase (2,3). Arginine, already present in the CNS, is converted, by NO synthase, to equal amounts of NO and citrulline (3).

In a recent study Dawson et al., reported that the NO antagonists N^{ω}-nitro-L-arginine (N-arg) and N^{ω}-monomethyl-L-arginine (M-arg) attenuated glutamate neurotoxicity in cortical cell cultures (4). In this study, NMDA neurotoxicity was associated with an increase in NO, which was likely mediated by the entry of calcium ions (4). Nitric oxide was also shown to mediate ischemic neuronal injury in rats (5). This study, conducted by Nowicki et al., showed that N-arg, a competitive inhibitor of NO synthase, reduced the volume of infarction (5). In a contrasting study by Demerle-Pallardy et al., the NO pathway was not involved in the neurotoxicity in the brain because the production of cyclic guanosine monophosphate (cGMP) by glutamate was totally blocked by the NO synthase inhibitors with no attenuation of neuronal injury (6). In another study conducted by Manzoni et al., nitric oxide was shown to protect against NMDA induced currents and the associated increase in intracellular calcium (7). Manzoni tested the effects of NO-induced ionic fluxes on NMDA by using drugs that produced NO. These drugs all blocked NMDA-induced currents.

The conflicting evidence suggests that more research needs to be done to determine the precise role of NO in the CNS. The discrepancy in results may be due to different methods, procedures, or distinct origins of neuronal cells. It is worthwhile to develop a detailed understanding of

the precise role of NO in NMDA neurotoxicity. This information could have implications to limit stroke-related brain damage. We performed this study in neuronal cell cultures to assess the role of nitric oxide in NMDA neurotoxicity.

MATERIALS AND METHODS

<u>Cell culture:</u> Cortical cell cultures consisted of both neuronal and glial cells. Cells were obtained according to the method of Regan et al. (8). Neuronal cells were obtained from fetal Swiss-Webster mice at 15-17 days gestation. The neocortex was removed, minced, and placed in media containing 0.08% acetylated trypsin at 37^{o} C for one hour. After centrifugation, the cells were placed in plating media consisting of Eagle's minimal essential media (MEM), 10% heat-inactivated horse serum, 10% fetal bovine serum, glutamine (2 mM), and glucose (21 mM). The cells were triturated through a flamed Pasteur pipette, diluted in plating media, and plated on pure glial cultures. The cells were plated in 15 mm multiwell plates (2.5 x 10 $^{-5}$ cells/well). The cultures were maintained at 37^{o} C in a 5 % CO^{2} incubator, and the media was changed twice weekly with growth media lacking fetal serum.

Glial cultures were obtained from neonatal Swiss-Webster mice. The dissection and dissociation was the same as the above method. The cells were plated on 15 mm Primaria (Falcon) multiwell plates at a

density of 0.5 hemispheres per plate. Glial cultures were used primarily as a feeder cell-layer for neurons.

Animal care and handling: Pregnant female Swiss-Webster mice were anesthetized with halothane and euthanized by means of cervical dislocation. The fetuses were removed, using sterile techniques, and immediately decapitated.

Cytotoxicity: Cells 15-19 days in vitro (DIV) were exposed to excitatory amino acids (EAA's) according to the methods of Regan et al. (8). Prior to exposure, the cells were washed with a HEPES (N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid])-buffered controlled salt solution (HCSS) to remove MEM. HCSS has the following composition: NaCl, 120 mM; KCl, 5.4 mM; $MgCl_2$, 0.8 mM; $CaCl_2$, 1.8 mM; HEPES, 20 mM; glucose, 15 mM. The cells were exposed to NMDA in HCSS for 5 minutes. The experimental solution was then washed with MEM. Cells were placed in an incubator for 20-24 hours. Long exposures to EAA's were performed in MEM with added glucose (25 mM).

LDH assay: Cell injury was quantified by measurement of the lactate dehydrogenase (LDH) released by damaged cells. Each LDH value was scaled to the mean value obtained by the control NMDA exposure. The LDH test was performed using the methods described by Regan et al. (8).

cGMP assay: After the application of NMDA 300 μm (5 min), the cell culture was placed in ice cold 65% ethanol for 5 min. The supernatant was extracted twice and lyophilized. The dried extracts were dissolved in the assay buffer, and the cGMP was measured with a cGMP enzyme assay kit purchased from Amersham.

NADPH-diaphorase staining: Control cultures and cultures exposed to EAA's were stained for reduced nicotinamide adenine dinucleotide phosphate diaphorase (NDP), described by Koh et al.(9). Cultures were fixed for 30 min in 4% paraformaldehyde at room temperature, and incubated in medium containing 1 mM NADPH and 0.2 mM nitro blue tetrazolium in 0.1 M Tris buffer (pH 8.2) at 37°C for 30 min to 1 hr. The staining action was ended by washing with water.

Reagents: All chemicals were purchased from Sigma (St. Louis, MO). The cGMP assay kit was obtained from Amersham (Amersham, UK).

RESULTS

By DIV 15, neurons in culture could be readily identified by their phase-bright cell bodies and extensive processes. The neurons were stained for NDP as an indirect measurement of cells containing NOS (9). Less than 1% of the neurons in these cultures were stained (Fig. 1).

Within minutes after exposure to NMDA 300 µM (5 min), neurons were acutely swollen. Over the next few hours, swollen neurons were beginning to deteriorate. At 24 hours the neurons were phase-dark and fragmented. The neurons were also releasing substantial amounts of LDH into the surrounding medium. Neuronal cell death was recorded 24 hours after NMDA (300 µm) exposure (Fig. 2). About 80% of the neurons were injured; the glial cell layer was left uninjured.

The simultaneous application of the NOS inhibitor 1 mM N-arg with NMDA 300 µM (5 min) did not provide protection against neuronal death (Fig 2). The application of 1 mM M-arg also did not provide protection. Approximately 75 % of the neurons were injured with each application. To further ascertain the role of nitric oxide in NMDA neurotoxicity, we applied reduced human A_0 hemoglobin (Hb), which binds NO, simultaneously with NMDA (300 µM). Hemoglobin provided no protection against NMDA-mediated neuronal injury (Fig. 3). Simultaneous application of the noncompetitive NMDA antagonist MK-801 completely blocked neuronal injury.

To determine if NO production was effectively blocked by the concentration of NOS inhibitors used, we assayed cGMP levels. The application of NMDA 300 µM (5 min) stimulated the formation of cGMP (Fig. 4). The formation of cGMP was completely blocked by the application of N-arg (1 mM) and Hb (500 µM). In fact, these cGMP levels were lower than baseline levels. Methyl-arginine, another NOS

inhibitor, less potent than N-arg (4), also partially blocked the formation of cGMP levels (Fig. 4).

DISCUSSION

Although Demerle-Pallardy used whole rat brain, our results were in agreement with their findings that NO was not shown to be involved in NMDA neurotoxicity (6). In contrast to Dawson et al., N-Arg and M-Arg (inhibitors of NOS) did not block NMDA-induced neuronal death (Fig. 2). Also, Hb (100 μm or 500 μM) did not protect against neuronal injury (Fig. 3). The discrepancy in results may be due to different methods, procedures, or distinct origins of neuron cells.

The cells exposed to NMDA 300 μM (5 min) resulted in approximately 80% neuronal death. A study by Dawson et al., suggests that N-arg and M-arg (NOS inhibitors) would block NO mediated neuronal death because they inhibit NOS (4). This inhibition would stop NOS from converting arginine into NO. Yet, in our system, N-arg and M-arg were not effective in blocking neuronal death. Approximately 70% neurons died with the simultaneous application of NMDA 300 μM (5 min) and the NOS inhibitors. Dawson et al's. evidence also suggested that Hb (which binds to NO) would block neuronal death (4). Our cultures were not protected against injury; approximately 80% neuronal death was recorded with this application. To prove that the concentrations of N-arg, M-arg, and Hb which we used were effective in

blocking the production of NO, we assayed the cGMP levels. The results suggest that N-arg, M-arg, and Hb were effective in blocking the formation of cGMP. These results suggest that NMDA-mediated neuronal death is independent of NO.

CONCLUSION

We showed that the nitric oxide synthase (NOS) inhibitors, nitro-arginine (N-arg) and methyl-arginine (M-arg) do not prevent NMDA neurotoxicity. Also, the application of hemoglobin (Hb) did not prevent neuronal injury. These data establish that NO does not mediate the neurotoxicity of NMDA. The discrepancy in results may be due to different methods, procedures, or distinct origins of neuron cells.

ACKNOWLEDGMENTS

Thanks to

Dr. Regan, Dr. Panter, Dr. Wheeler, COL Brown, SSG Thomas, Susan Siefert, Donna Shepard, and Andre Akers.

ANIMAL CARE AND HANDLING STATEMENT

The opinions and assertions contained herein are the private views of the authors and are not to be construed as official nor do they reflect the views of the Army or the Department of Defense. (AR 360-5)

The experimental studies of the author described in this report were reviewed and approved by the Institutional Review Committee/Animal Care and Use Committee at Letterman Army Institute of Research. The manuscript was peer reviewed for compliance prior to submission for publication. In conducting the research described here, the author adhered to the "Guide for the Care and Use of Laboratory Animals," DHEW Publication (NIH) 85-23.

REFERENCES

1. Zivin JA, Choi DW. Stroke Therapy. Scientific American. 1991;July:56-63.

2. Snyder SH, Bredt DS. Biological Roles of Nitric Oxide. Scientific American 1992;July:68-77.

3. Garthwaite J. Glutamate, nitric oxide and cell-cell signaling in the nervous system. TINS 1991;14:60-67.

4. Dawson VL, Dawson TM, London DE, Bredt DS, Snyder SH. Nitric oxide mediates glutamate neurotoxicity in primary cortical cultures. Neurobiology 1991;88:6368-6371.

5. Nowicki JP, Duval D, Poignet H, Scatton B. Nitric oxide mediates neuronal death after focal cerebral ischemia in the mouse. European Journal of Pharmacology 1991;204:339-340.

6. Pallardy CD, Lonchampt MO, Chabrier PE, Braquet P. Absence of implication of L-arginine/nitric oxide pathway on neuronal cell injury induced by L-glutamate or hypoxia. Biochemical and Biophysical Research Communications 1991;181:456-464.

7. Manzoni O, Prezeau L, Marin P, Deshager S, Bockaert J, Fagni L. Nitric oxide-Induced Blockade of NMDA Receptors. Neuron;1991:653-662.

8. Regan RF, Choi DW. Glutamate Neurotoxicity in Spinal Cord Cell Culture. Neuroscience.1991;43:585-591.

9. Koh J, Choi DW. Vulnerability of Cultured Cortical Neurons to Damage by Excitotoxins: Differential Susceptibility of Neurons Containing NADPH-Diaphorase. The Journal of Neuroscience 1988;8:2153-2163.

10. Snyder SH. Nitric oxide: First in a New Class of Neurotransmitters? Science 1992;257:494-496.

Fig. 1, NADPH-diaphorase neuron stain. Three NDP neurons with darkly stained cell bodies can be seen against a background of other largely unstained neurons and glia.

Fig. 2, Protection provided by N-arg (1 mM) and M-arg (1mM). Amount of LDH present in bathing media was measured 24 hours after the application of NMDA 300 µm (5 min) and antagonists (mean ± S.E.M., n=4 cultures at each point). LDH release was scaled to the mean value evoked by exposure to 300 µm NMDA, 24 hrs (=100).

Fig. 3, Protection provided by Hb (100 µm and 500 µm). Amount of LDH present in bathing media was measured 24 hrs after the application of NMDA and antagonists (mean ± S.E.M., n=4 cultures at each point). LDH release was scaled to the mean value evoked by exposure to 300 µm NMDA, 24 hrs (=100).

Fig. 4, Formation of cGMP after the application of NMDA with or without N-arg 1 mM, M-arg 1 mM, or Hb 500 µm. Data are means ± S.E.M.

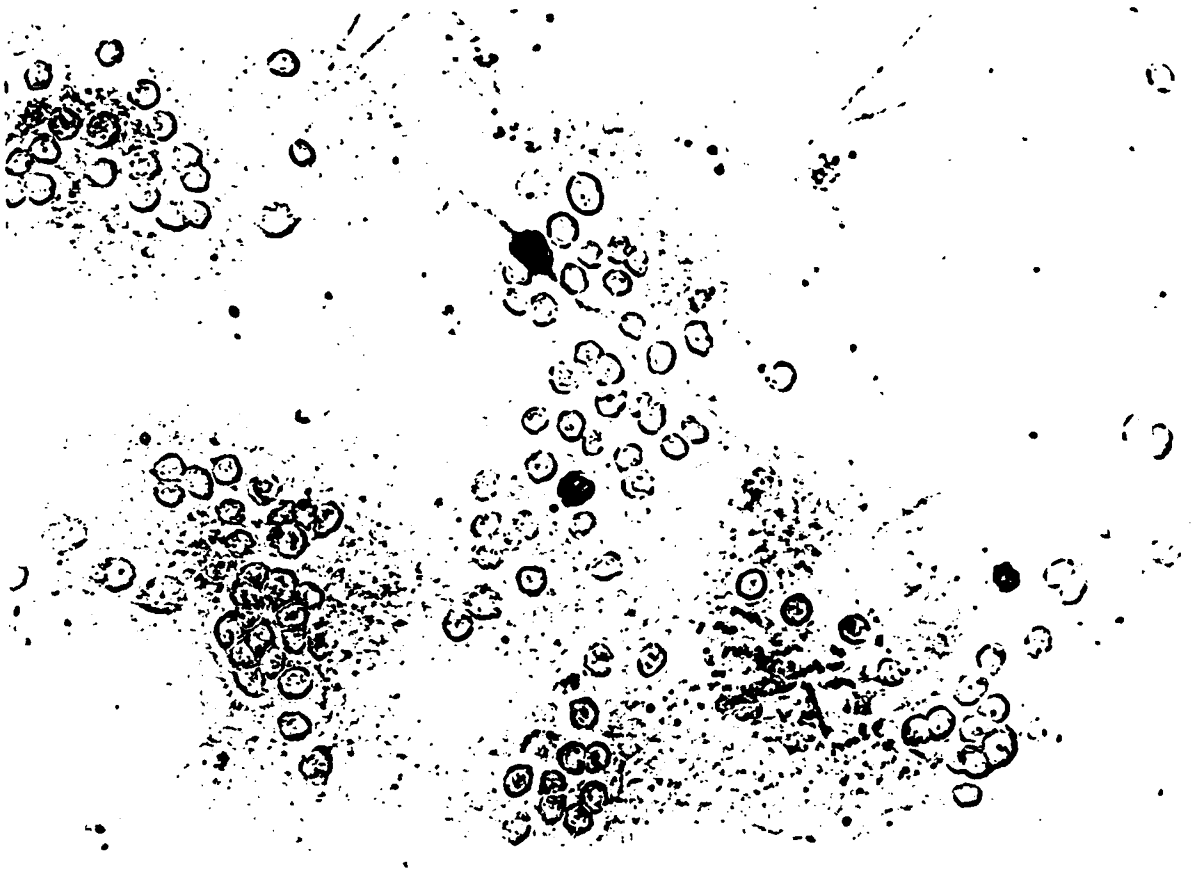


FIGURE 1

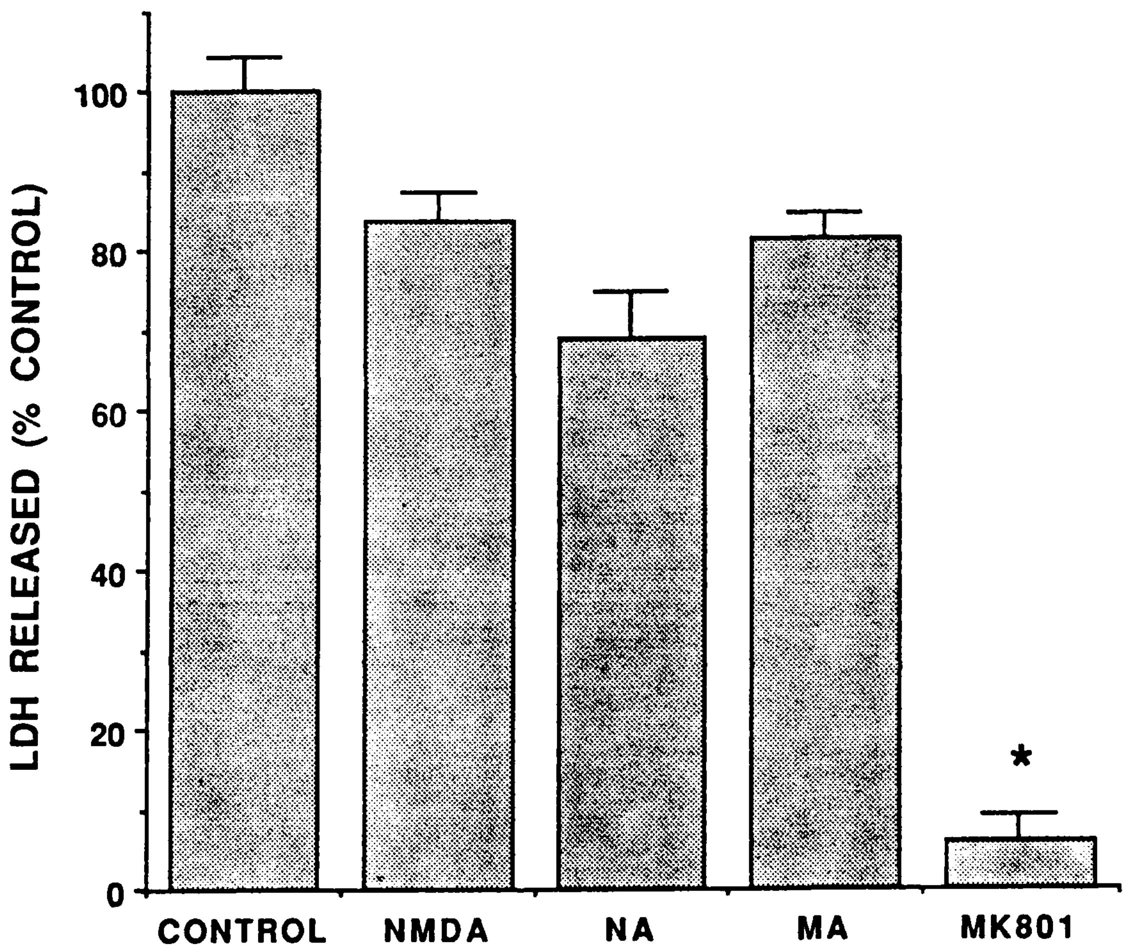

LDH RELEASED (% CONTROL)
100
80
60
40
20
0
*
CONTROL
NMDA
NA
MA
MK801

FIGURE 2

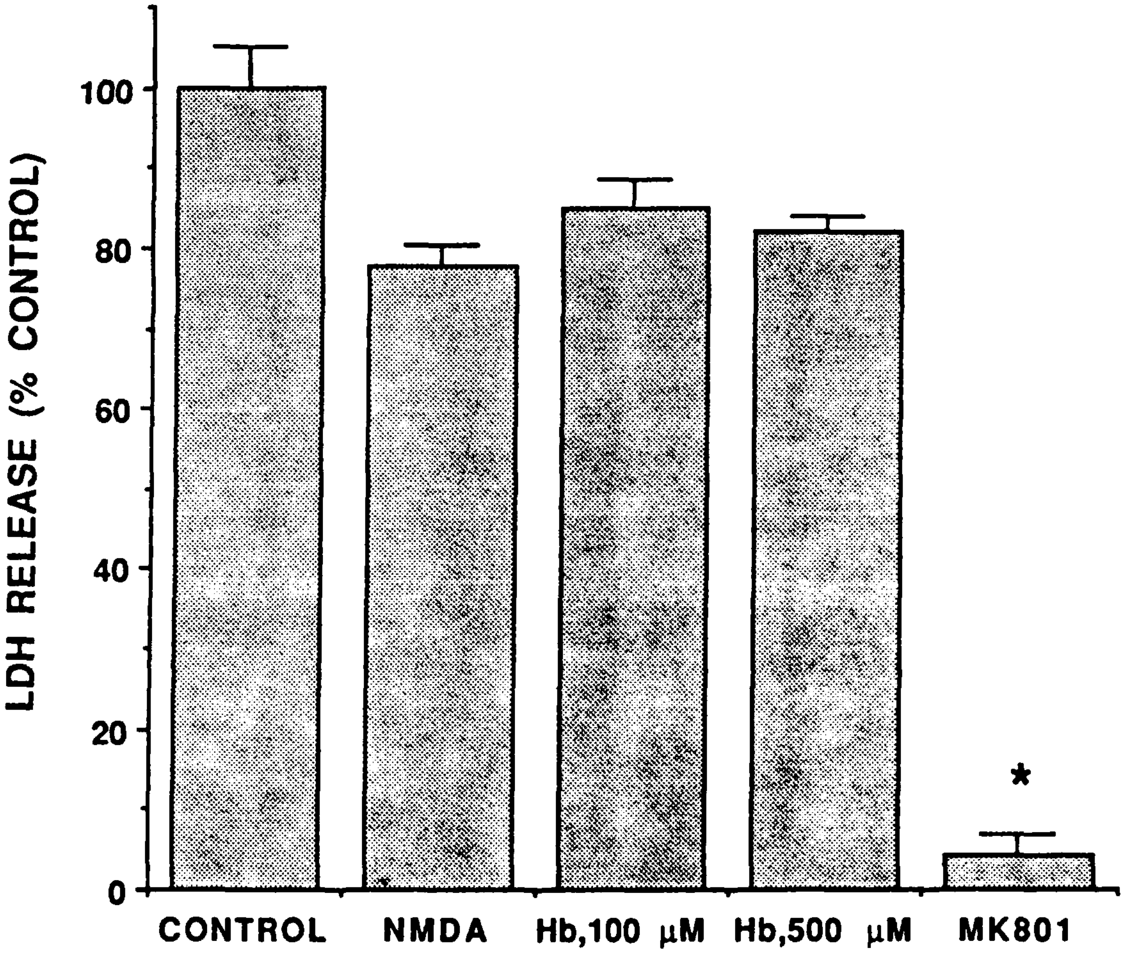
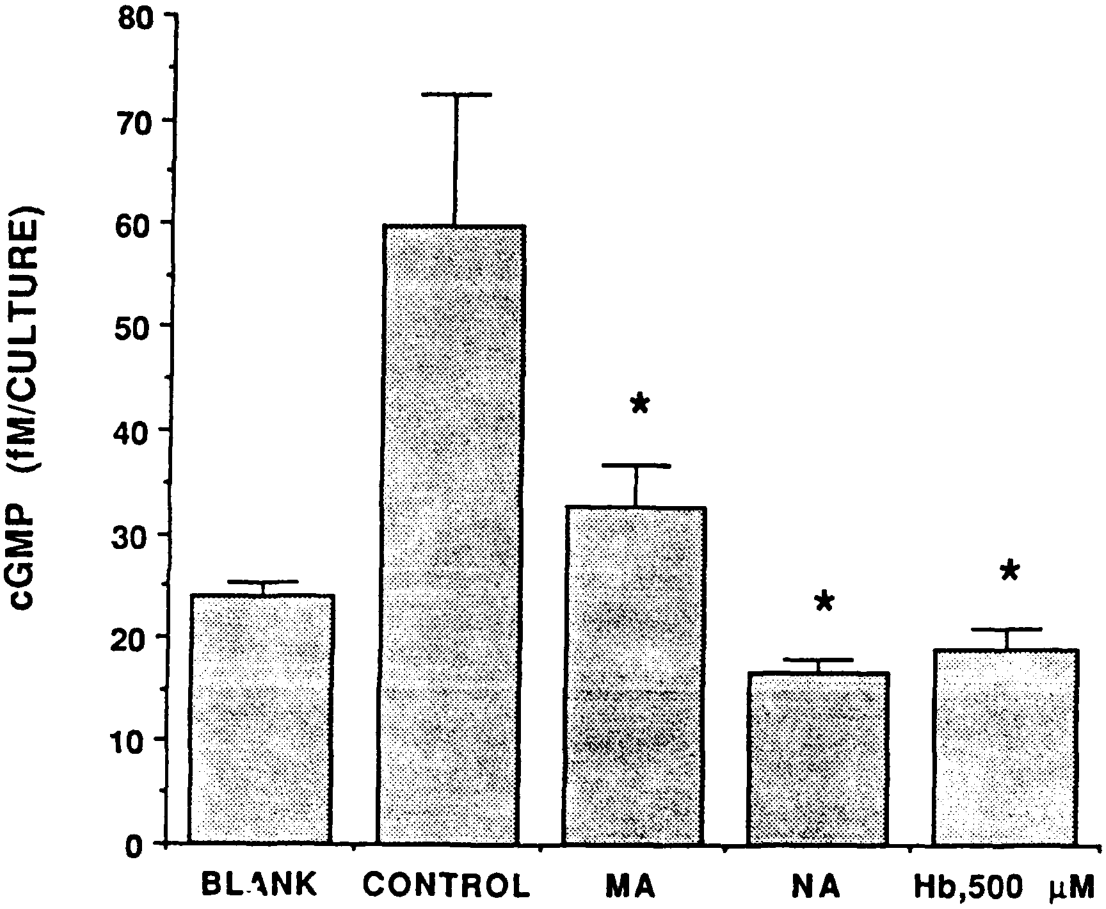

LDH RELEASE (% CONTROL)
100
80
60
40
20
0
*
CONTROL
NMDA
Hb,100 μM
Hb,500 μM
MK801

FIGURE 3



cGMP (fM/CULTURE)
80
70
60
50
40
30
20
10
0
*
*
*
BLANK
CONTROL
MA
NA
Hb,500 μM

FIGURE 4

Thornton Samuel Mu

Lowell High School

August 12, 1992

Letterman Army Institute of Research

Mentor - Michael A. Dubick, Ph.D.

Pyridoxine deficiency and wound healing in rats:

Assessment of antioxidant enzymes and

immunological status

Abstract

Many nutrients are involved in the complex process of wound healing. Of the vitamins, pyridoxine (vitamin B_6) deficiency has resulted in impaired healing, most likely due to its effect on protein metabolism. This study examined the effects of pyridoxine deficiency on wound healing and its effects on acute phase proteins, immunoglobulins, and antioxidant enzymes in rats. The results showed that despite pyridoxine deficiency, the animals in each dietary group responded the same toward injury.

Pyridoxine deficiency and wound healing in rats: Assessment of antioxidant enzymes and immunological status -- Thornton Mu

Wound healing is a complex process that takes place in many stages (1) and requires the presence of many important hormones, nutrients, and proteins, such as acute-phase proteins, immunoglobulins, and proteins that regulate dangerous oxidants (2-5). Free radicals formed by these oxidizers can cause serious cell injury, such as lipid peroxidation (6).

Among the nutrients, pyridoxine (vitamin B_6) plays quite an important role in the synthesis and maturation of connective tissue (7). Consequently, pyridoxine deficiency can affect normal wound healing (8). Pyridoxine also possesses an antioxidant function that prevents damage done by free radicals (9), functions as a coenzyme in the metabolism of amino acids (10), and is important for normal immune function (11). Because proteins, such as immunoglobulins, antioxidant enzymes, and acute-phase proteins, are composed of amino acids, it is assumed that a deficiency in vitamin B_6 would adversely affect production of these proteins.

We proposed that pyridoxine deficiency would impair the ability to respond to injury. In this study we examined the effects of various stages of pyridoxine deficiency on the status and presence (activity) of

antioxidant enzymes and on the concentrations of certain acute-phase proteins and immunoglobulins in an incisional wound model in the rat. A better understanding of the role of pyridoxine could lead to improved therapeutic regimens or preventive measures, thus reducing mortality and pain for the injured subject.

Materials and Methods

Experimental protocol

Young adult, female Long-Evans rats, initially weighing 175-200g were employed in these studies. A total of twenty-four rats were used in the study. The rats were randomly assigned to four groups of six rats. The first group was fed a diet devoid of (deficient) vitamin B_6. The second group was fed a diet with 0.25 mg vitamin B_6 per kg. The third group was fed a diet with 1 mg/kg vitamin B_6. The fourth group was fed a control diet with 7 mg/kg vitamin B_6. All four groups were fed their respective diets for a period of five weeks. Three rats within each group underwent surgery in which an incision was made through the full thickness of the skin on the dorsal side. The rats recovered during one week after the surgery and were fed their respective diets during the recovery period.

Biochemical Studies

At the end of six weeks, the rats were overdosed with pentobarbital (120mg/kg). Blood was withdrawn by cardiac puncture into citrated tubes and plasma was recovered by centrifugation for further evaluation of vitamin B_6 levels, immunoglobulin G and M, albumin, and fibrinogen. Liver, kidney, and brain were quickly removed, snap frozen in liquid nitrogen, and stored at -70°C in a biological freezer until assayed.

In preparation for assays to determine glutathione peroxidase, glutathione reductase, superoxide dismutase, and protein content, samples of kidney, liver, and brain were homogenized separately using the Polytron homogenizer (Brinkmann Instruments, Westbury, NY) in a homogenizing buffer composed of 0.25M sucrose and 10mM Tris-HCl, pH 7.4 for ten seconds. Using a Branson sonifier cell disruptor (Branson Ultra Sonic, Danbury, CT), the samples were sonicated three times for a period of five seconds each. The samples were centrifuged for thirty minutes at 10,000g in a RC-5 Superspeed Refrigerated Centrifuge (DuPont Instruments, Newton, CT).

The samples were tested for glutathione peroxidase by using hydrogen peroxide as a substrate (12) and tested for glutathione reductase activity (13) by measuring the disappearance of beta-nicotinamide adenine dinucleotide phosphate (beta-NADPH) using the

Beckman DU-70 Spectrophotometer (Beckman instruments, Fullerton, CA).

Total superoxide dismutase (SOD) (12) activity was measured by the ability of the enzyme to inhibit auto-oxidation of pyrogallol using the Beckman DU-70 Spectrophotometer. Manganese SOD activity (12) was measured under the same conditions except the buffer contained 1mM KCN. CuZnSOD was calculated by subtracting MnSOD activity from the total SOD activity.

Immunoglobulin G and M (IgG and IgM, respectively) concentrations in plasma were determined in diffusion plates (Kallestad, Chaska, MN) by double immunodiffusion (14). The results were recorded after 24 and 48 hrs incubation but only the 48 hr values were used according to standard protocol in clinical laboratories. Ten μL of antibody was placed in the center well with dilutions of plasma placed in the outer wells. For IgM, neat through 1/32 dilutions were used and for IgG, 1/32 to 1/1024 dilutions were used.

Fibrinogen and albumin concentrations were determined by rocket immunoelectrophoresis (150 using the Bio-Rad horizontal electrophoresis cell (Bio-Rad laboratories, Richmond, CA). Albumin concentrations were determined from standards, while fibrinogen concentrations were calculated as arbitrary units based on dilutions of normal rat plasma.

Protein content was determined with a commercially available kit (Bio-Rad, Richmond, CA) using the Beckman Du-70 spectrophotometer.

Statistical Analysis

Data were analyzed by two way ANOVA with injury and diet as factors. Significant differences were further evaluated by the Newman-Keuls method for multiple comparisons. A $p<0.05$ was considered statistically significant.

Animal Use Statement

Animal quarantine and prior care were performed in accordance with SOP# OP-ARG-4 and OP-ARG-40 and conforms to the provisions of the NIH as stated in the Guide for the Care and Use of Laboratory Animals (NIH publication #85-23).

Results

Pyridoxine status

Tests to determine pyridoxine status in rats showed that rats with no vitamin B_6 in their diets were indeed deficient in vitamin B_6 whereas the control rats with 7mg/kg of pyridoxine had normal vitamin B_6 concentrations in their plasma. The other two groups had intermediate vitamin B_6 levels in their plasma (data not shown).

Acute-phase proteins (Table 1)

Concentrations of fibrinogen were not significantly affected by either diet or injury whereas albumin concentrations were significantly lower in rats with injury (p-0.0023) and those with pyridoxine deficient diets ($p=0.0002$).

Immunoglobulin G and M

Concentrations of IgG and IgM were not significantly different among the dietary groups and injury versus noninjury groups, but were relatively constant.

Antioxidant enzyme status (Table 2)

Activity of glutathione peroxidase per gram of tissue (GPx/g) was significantly higher ($p=0.0240$) in the livers of rats deficient in vitamin B_6 compared to controls. However, in brain and kidney samples, GPx activity was not significantly affected by the different vitamin B_6 diets. in all dietary groups, GPx activity from brain samples from injured rats showed a significant decrease ($p=0.0229$) compared to their uninjured counterparts whereas in the liver and kidney samples, a nonsignificant trend of decrease was noted.

In the rats that were deficient in vitamin B_6, activity of glutathione reductase per gram of tissue (GR/g) was significantly lower

(p=0.0278) in liver samples, but significantly higher (p=0.0034) in brain samples compared to controls. Activity of GR/g in kidney was not significantly affected by diet or injury.

Differences in manganese superoxide dismutase activity per gram of tissue (MnSOD/g) were not significant among the diet and injury status of the three tissue groups.

However, activity of copper zinc superoxide dismutase per gram of tissue (CuZnSOD/g) was significantly lower (p=0.0391) in kidney samples from rats fed deficient pyridoxine diets but was significantly higher (p=0.0390) in brain samples with the same deficient diets compared to controls. Liver sample activity remained non-significant. In terms of injury status, CuZnSOD/g activity was non-significant among the three tissues.

Discussion

It has already been shown that vitamin B_6 affects protein metabolism (10), and in our studies, protein levels of rats with diets deficient in vitamin B_6 were significantly reduced (data not shown). However, similar to studies by Schaeffer et al. (16), body weight of all rats remained relatively constant despite the varying amount of pyridoxine in their diets (data not shown). Therefore, to insure more

stable readings, activity of the antioxidant enzymes measured were expressed per grams of tissue rather than grams of protein.

Our results have shown that there were few significant decreases in enzyme activity due to changes in pyridoxine diet or injury status. One could possibly assume that with a shortfall of protein, amounts of other proteins and enzymes could also decrease. Yet this assumption did not hold for the enzymes and proteins measured in our studies because fibrinogen, IgG, IgM, GPx, GR, MnSOD, and CuZnSOD concentrations did not decrease significantly. Instead, in tissues in which antioxidant enzymes activity was significantly affected by diet, the values were usually higher in the deficient group than in the controls.

One possible explanation for these findings is that the one week allowed for recovery from surgery may have been enough time to synthesize the enzymes and proteins listed above. In addition, a previous study done by Black et al. showed that rats with pyridoxine-deficient diets maintained a vitamin B_6 reserve in their gastrocnemius muscle (17). Therefore, although the plasma revealed a pyridoxine deficiency, the rats may have been relying on this storage to provide tissues with enough pyridoxine to synthesize the needed enzymes and proteins to respond to the stress of the injury induced.

Albumin levels, on the other hand, showed a significant decrease

both in the vitamin B_6 group and the injured group. Albumin in plasma was affected by pyridoxine deficiency, but the reaction of albumin as an acute-phase protein following injury was not impaired.

In conclusion, pyridoxine deficiency did not significantly affect the rat's response to surgically-induced wound. The antioxidant status of the tissues examined in the rats remained at control level, or higher, and the plasma concentrations of immunoglobulins measured were unaffected by the pyridoxine deficiency. Also, the acute-phase protein response to injury was not adversely affected by the pyridoxine deficiency. More research is needed to gain a better understanding of the effects of vitamin B_6 in the realm of wound healing.

Acknowledgements

I would like to thank Dr. Michael Dubick and Dr. X.Q. Yuan for their sharing their vast knowledge, learning, and experience with me; Dr. Virginia Gildengorin for assisting with the statistical analyses; Ms. Susan Siefert for helping me prepare this paper; and the Military Trauma Research Division and COL Brown, MAJ McCollum and Mr. William Ward of the Command Division at the Letterman Army Institute of Research for giving me this tremendous opportunity.

REFERENCES

1. Hotter, AN. Physiologic aspects and clinical implications of wound healing. Heart & Lung 1982;11:522-530.

2. Kushner, I. The Acute Phase Response: An Overview. Methods in Enzymology 1988;163:373-383.

3. Ritzman SE, Daniels JC. Laboratory Notes: Serum Proteins, Somerville, NJ: Behring Diagnostics, Nov. 1-28, 1973.

4. Murphy FA, Osebold JW, Aalund O. Kinetics of the antibody response to Anaplasma marginale infection. J Infect Dis 1966;16:99.

5. Harris ED. Regulation of antioxidant enzymes. The FASEB J 1992;6:2675-2683.

6. Slater TF. Free-radical mechanisms in tissue injury. Biochem J 1984;222:1-15.

7. Tinker D, Rucker RB. Role of selected nutrients in synthesis, accumulation, and chemical modification of connective tissue protein. Physiol Rev 1985;65:607-657.

8. Lakshmi AV, Diwan PV, Bamji MS. Wound healing in pyridoxine deficiency. Nutri Res 1988;8:1203-1206.

9. Wills ED. The role of dietary components in oxidative stress in tissues. In: Oxidative Stress, H Sies, ed., Academic Press, New York, 1985, pp. 197-218.

10. Dubick MA. Interactions of vitamin B_6 and xenobiotics. In: Nutritional Toxicology, Hathcock JN, ed., Academic Press, Orlando, 1989;3:97-121.

11. Chandra RK, Sudhakaran L. Regulation of immune responses by vitamin B_6. In: Vitamin B_6, Dakshinamurti K, ed., Academic Press, New York, 1990, pp. 404-423.

12. Zidenberg-Cherr S, Han B, Dubick MA, Keen CL. Influence of dietary-induced copper and manganese deficiency on ozone-induced

changes in lung and liver antioxidant systems. Toxicol Lett 1991;57:81-90.

13. Cohen MB, Duvel DL. Characterization of the inhibition of glutathione reductase and the recovery of enzyme activity in exponentially growing murine leukemia (L1210) cells treated with 1,3-bis(2-chloroethyl)-1-nitrosourea. Biochem Pharm 1988;37:3317-3320.

14. Clausen J. Immunochemical techniques for the identification and estimation of macro-molecules. New York: American Elsevier Publishing Company, Inc., 1969.

15. Laurell, CB. Electroimmunoassay. Scand J Clin Lab Invest 1972;29(suppl. 124):21-37.

16. Schaeffer MC, Sampson DA, Skala JH, Gietzen DW, Grier RE. Evaluation of vitamin B_6 status and function of rats fed excess pyridoxine. J Nutri 1989;119:1392-1398.

17. Black AL, Guirard BM, Snell EE. The behavior of muscle phosphorylase as a reservoir for vitamin B_6 in the rat. J Nutri 1978;108:670-677.

David M. Dahle

Tamalpais High School

August 5, 1992

Letterman Army Institute of Research

Mentor - Kim Vandegriff, Ph.D.

Data Acquisition for Experiments in Hemoglobin Oxygen Binding

ABSTRACT

This report presents an Assembly Language program that was designed to collect experimental data in the form of analog voltage signals and convert them into digital values for computer storage and analysis. These voltage signals represent the concentration of oxygen in a reaction cell for an experiment which tests the function of hemoglobin by measuring the color change of the hemoglobin solution as it transforms from deoxyhemoglobin to oxyhemoglobin upon the addition of oxygen. The program interfaces the experiment with an analog-to-digital converter that improves the accuracy of the oxygen concentration measurements. The program also incorporates specific timing sequences to allow synchronous data acquisition. The Assembly Language program employs user-friendly interfacing and several automated features that result in saving of time and effort on the part of the researcher.

DATA ACQUISITION FOR EXPERIMENTS IN HEMOGLOBIN OXYGEN BINDING

David M. Dahle

This project in computer science was designed to translate experimental data signals into digital files for data analysis. The data translation procedure will be used in experiments to aid in the development of a hemoglobin-based blood substitute.

The biochemical experiment involves testing the structure and function of hemoglobin by measuring its transition from deoxyhemoglobin to oxyhemoglobin as oxygen is added and the protein changes color from purple to red. The change in color of hemoglobin is measured optically by rapid-scanning spectrophotometry, which records the amount of light absorbed by hemoglobin at each wavelength in the visible spectrum from 400 to 800 nanometers. The absorbance values for each wavelength are measured as rapidly scanning monochromatic light is transmitted through a reaction chamber containing the hemoglobin solution. Oxygen concentrations are recorded simultaneously as a voltage level, which is produced by an electrode fitted in the reaction cell. This new methodology measures the

change in the hemoglobin spectrum at 5 scans per second.

This project involves the aspect of the experiment that records the oxygen concentrations in the reaction cell containing the hemoglobin, which is done simultaneously with the recording of the absorbance values. The program that records the oxygen concentrations receives this information as an analog voltage signal. It converts the voltage to digital values using an analog-to-digital converter. It synchronizes its operations with the recording of the absorbance values using a digital signal sent by the spectrophotometric system. The oxygen concentration values are then combined with the absorbance values and analyzed. Figure 1 shows these data graphed in 3-dimensions. The graph begins with deoxyhemoglobin and ends with oxyhemoglobin.

This project was undertaken to upgrade an outdated data acquisition system in an effort to improve the accuracy of the oxygen concentration readings. Other goals of the project were to incorporate a user-friendly interface and a flexible design so that the program would be easier to operate, making data

acquisition as simple as possible. The new data acquisition system arrived in the laboratory just before I did, so I began the project by first installing the computer board, fabricating new cables, and then developing the program.

EQUIPMENT USED

COMPUTERS

MS-DOS 80286.

CompuAdd Tower MS-DOS 80486.

GoldStar MS-DOS 80386.

Digital Equipment Corporation network system and software.

PROGRAM DEVELOPMENT

WordPerfect Version 5.1.

WordPerfect for Windows Version 5.1.

Microsoft Windows Version 3.1.

Microsoft DOS Version 5.0.

WordStar Professional Version 5.0.

COMPILERS/ASSEMBLERS

Borland International Turbo Assembler Version 1.0 and utilities.

Microsoft C Optimizing Compiler Version 5.0, Version 5.1 and utilities.

EXPERIMENTAL APPARATUS

LT Quantum 1200 Spectrophotometric System (LT Industries Rockville, Maryland) is used to make the spectral measurements of the hemoglobin.

YSI Model 5300 Biological Oxygen Monitor System (Yellow Springs Instrument Co., Inc. Yellow Springs, Ohio) is used to create the oxygen voltage concentration signal.

Analog Connection MINI-16 Data Acquisition and Control System Analog to Digital Converter (Strawberry Tree Computers, Inc. Sunnyvale, California) is used to convert the analog signal to a digital value so it can be stored and used in a computer.

EXPERIMENTAL METHOD

The experiment has two components which run synchronously. (See Figure 2). The first component is the spectrophotometric system which makes the absorbance value readings. The software for this system controls the rapid-scanning spectrophotometer and collects data from it. The data are recorded as absorbance values for each wavelength in the visible light spectrum from 400 nanometers to 800 nanometers. Absorbance values at each wavelength are measured as

rapidly-scanning monochromatic light is transmitted through the hemoglobin solution. The system also signals the oxygen concentration recorder via a TTL (Transistor to Transistor Logic) pulse, so the oxygen concentration recordings can be synchronized with the spectral scans. This is a digital signal that represents two states, on or off, or, in this case, "record" or "don't record" oxygen concentrations.

The second component of the experiment measures the amount of oxygen in the reaction chamber simultaneously with the spectral readings. The system begins with a Clark-type, fast response polarographic electrode that is fitted in the reaction cell containing the hemoglobin solution. It generates an analog voltage signal based on the concentration of oxygen in the reaction cell. The signal goes to an oxygen monitor which amplifies and calibrates it, then to a filter to reduce line noise. A voltmeter gives a display of the approximate voltage signal. The signal then travels to the analog-to-digital converter in the computer. This converter has 8 analog input/outputs and 16 digital input/outputs. An analog port is used to read the oxygen voltage concentration signals and

convert them to digital values which only then can be stored in the computer. A digital port is used to read the TTL signal so that the oxygen concentration recorder will run synchronously with the spectrophotometric system.

PROGRAM DESCRIPTION

The program relies on a device driver to run the hardware, so that the driver must be installed before the program can be run. When the program starts it verifies that the driver has been installed. If it has not, the program displays an error message, waits for the user to hit any key, then exits. If the device driver has been installed, the program accesses the functions by placing parameters on the stack and calling interrupt vector 60.

After the test for the presence of the software, the program loads a default configuration file which contains the settings for all the configuration fields and the date they were saved. This date is used by the *Automatic File Names* feature. This feature enables the program to automatically create names for the files based on the date and the current experiment number. It creates file names by generating an 8-character

numerical string, the first two characters being the month of the year, the next two the day of the month, the next two the last two digits in the year, and the last two the experiment number (*i.e.*, 12259250 for December 25, 1992, experiment number 50). After each experiment is run without errors, the program automatically increments the experiment number. When a configuration file is written, the date to which the experiment number corresponds is also saved. When the file is loaded again, the program compares the date in the file with the current date. If they are the same, it restores the experiment number from that file, if they are different, it resets it to 1. This allows the experiment number to act as an automatic counter for the number of experiments run in a day, even if the program is run several separate times in the same day.

After the configuration file is loaded, the user is given a list of several functions which can be performed. The user makes selections from these by using the left and right arrow keys. Each item will become highlighted as it is selected. The user can execute the currently highlighted item by hitting the return key. If the user presses the up or down keys

he/she can scroll through the configuration parameters. Highlighted configuration items can be toggled with the return key. String entry fields are used to read file names from the user and function in the same way as any other string input field.

The *Average File Name* field specifies the file in which the averages of all the readings for each data set will be written. The *Data File Names* field specifies the name for the data files in which every reading from each set will be written. An extension number corresponding to the current set is used so the data for each set can be distinguished, and any extension given will be replaced. If no file name is given for either of the two file name fields, then no file will be created. The *Experiment Number* field specifies the number of the current experiment, which is used by the *Automatic File Names* feature. This number is ignored by the program if this feature is turned off. The *Automatic File Names* field allows the user to turn on or off the automatic file naming feature. The *Print Elapsed Time* field allows the user to specify if he/she wants the program to compute the length of time that the TTL pulse was active and the

data rate in Hertz (Hz). The *Analog Input Range* field is used by the device driver to indicate the projected voltage range of the input signal. The *Analog Input Resolution* field is also used by the device driver to determine the precision of the readings. This field is also used to set the speed at which data are sampled. The less precision specified, the faster the data are converted.

The first item from the list of functions is the *Begin* item which is used to start data acquisition. Once started, the function list will be overwritten by a message informing the user that he/she can end data acquisition by pressing any key. If any file or memory errors occur, then an error message is displayed and acquisition is aborted. If the *Automatic File Names* feature has been activated, then the file names are given just before acquisition begins.

Acquisition begins by waiting for a drop in the TTL line from the spectrophotometric software. This line is connected to one of the digital input ports on the analog-to-digital converter. It remains in an active state (low-voltage mode) until the specified number of scans has been completed. When spectral data

collection is finished, the signal returns to its inactive state (high-voltage mode). The program senses the change in the TTL signal and stops recording voltage readings. An average of all of the readings in each set is computed and written to an output file, if a file name was given in the *Average File* field. If the user specified a file name in the *Data File Names* field, then each reading in the set will be written to disk using the name given plus an extension corresponding to the current set number. This process is repeated until the user cancels the operation by hitting any key, allowing sets of readings to be taken.

The next item in the function list is *Instant* which continuously reads both the analog voltage and the TTL line state so that the user can verify the connections before an experiment begins. This can be done by comparing the voltage readings with those on the voltmeter.

The *Load* function enables the user to read a configuration file with a name other than the default name. A string input field appears just below the function list in which the user can enter the new file name. He/she can abort by hitting the escape key.

When done, the user presses the return key to write the file.

The *Save* function allows the user to write a configuration file with a name other than the default one. A string input field appears and functions the same as the *Load* function.

The last item in the function list is the *Exit* option. When this function is selected, the program writes the current configuration settings to the default configuration file, then returns to the parent process, normally the MS-DOS command prompt.

EXPERIMENTAL DATA

Figure 3 shows the fractional saturation of hemoglobin calculated as a function of the partial pressure of oxygen. The fractional saturation of hemoglobin (Hb) is computed using the following formula:

Fractional Saturation = %oxyHb / (%oxyHb + %deoxyHb).

DISCUSSION

The program went through two versions. The first was written in the C Programming Language (see Appendix A for source listing). The second version was written almost entirely in 80286 Assembly Language, only

functions which involve the processing of floating-point variables were written in C (see Appendix B for source listing). The rewriting of the program solved several problems and allowed me to discover better solutions for old ones.

A few improvements were also made from the old system which was in place previously, beyond the increased accuracy provided by the new data acquisition system. These include the use of 8-byte doubles instead of 4-byte floats in computing the averages. This increased the number of decimal places used in the calculation and, therefore, the precision of the computation.

A major difference was discovered between the new data acquisition and the old one. In the old system, the converter would start recording as usual with the TTL pulse. Samples were then taken at a specified rate until the TTL pulse went inactive. The number of readings might vary if the timing of the spectrophotometric system was not always accurate, but the samples were always taken at the same rate and within the correct time period. A specific sampling frequency in Hz could be approximated by setting the

rate at which readings were taken and by computing the time that spectrophotometric system would take, which is known. This was required so that a sampling frequency could be set to minimize line noise.

On the new system there is no direct way to set the sampling frequency. It can only be set indirectly through the resolution of the analog-to-digital conversion. It also has no feature to automatically start or stop recording with a TTL signal. This problem at first led us in the direction of attempting to compute the rate at which samples would be taken based on the resolution and the clock speed of the computer. We thought we could then set the number of readings so that the sampling time would cover the same time that the spectrophotometric system was recording, which could be computed as before. But problems were found with this solution. The first was that this method left no room for error on the part of the spectrophotometric system in terms of the time it takes in making its measurements. If for some reason it took more or less time, the oxygen recorder would have no way of knowing this, and the data would not all be from the same time period. The manual for the new converter

also stated that the rate of data conversion would increase with the clock speed of the computer, but it gave no further information and no method for calculation of this function. A formula for computing the rate was found in an example program, but worked only for 4.77 MHz machines, and we are using a faster machine.

Because the issue of primary importance is to record the data simultaneously, a trade off was made and a method similar to the old one was used. The data recording would stop only when the TTL pulse returned to an inactive state. This increased program overhead, and the sampling frequency was reduced by approximately 20% because the TTL pulse had to be checked between each reading. This was an acceptable solution because only the averages of the readings in a set are commonly used during data analysis. During testing, a resolution was found that would produce a Hz close to that used in the old system (*i.e.*, 120 Hz), so the new solution worked, though not elegantly.

The first version of the program uses the first method described to solve the timing problem. It is capable of taking more samples per set, but the timing

is unsure. The second version uses ~~the second method,~~ it takes fewer readings per set, but the timing is always correct. Neither version is capable of implementing a specific Hz, due to the hardware limitations.

There is also a minor concern in computing the elapsed time. The clock used to read the time updates only 18.2 times per second, which allows for approximately a 5.4-hundredths of a second variance. But the values computed from this time are only for reference and are not used in any other calculations, so this may not be of much consequence.

A bug was found in the driver routines which caused the program to crash under certain conditions. Whenever a certain function in the driver was called and the direction flag of the processor was set, the driver ended up attempting to execute instructions in the interrupt vector table, the beginning of memory. The direction flag is used by the processor during string instructions to determine the direction of the strings in memory, either forwards or backwards. Normally this flag is set properly before any string instruction is used and does not need to be in any

predetermined state, but the device driver function does not reset this flag. A solution was found by inserting an instruction to clear this flag before calling any device driver function. Forgetting to reset the direction flag is a common programming error on the 80x86.

IMPLICATIONS

This project has accomplished its two objectives. The first was to improve the accuracy of data acquisition, which is mostly a result of the new data acquisition system. The second was to make the acquisition of data as simple as possible for the user. This has been accomplished by designing a user-friendly interface and automating as much of the process as possible, leaving more time and energy for the experiment. Much improvement has been made in this area as compared with the old program. As a result, the data will not only be more accurate but easier to collect.

CONCLUSIONS

This project only took five of the eight weeks that I spent as an apprentice at LAIR. I also wrote a program to process the spectral data from the

spectrophotometric system (see Appendix C for source listing). The original two programs run on a VAX network system because they require such a large amount of memory. Because the experiment is going to be moved next year to a location where a network may not be available, a program was needed that would not require the network. The new program uses disk buffers instead of memory and is therefore much slower, but if a RAM disk is used, program performance is greatly enhanced. At any rate, slowly acquired data are better than no data at all.

Many other smaller tasks were undertaken along the way. These include but are not limited to the installation and trouble shooting of several programs, the optimization of (though not understanding of) a Fortran program that models oxygen diffusion in and out of red blood cells, and the successful installation of OS/2 on one computer. One of the most significant accomplishments was learning Intel 80x86 Assembly Language during the first weeks, which enabled the second version of the data acquisition program to be written.

ACKNOWLEDGEMENTS

I especially wish to thank my mentor, Dr. Kim Vandegriff, Ph.D., for accepting me into this program and for taking so much time away from her work to assist me.

I would like to thank Yvonne Le Tellier for helping me so closely during the first days, when I most needed it.

I would also like to thank Turney Steward for all the insightful discussions on programming languages and philosophies he had for me.

Finally, I would like to express my gratitude to Susan E. Siefert for her assistance in the writing and editing of this paper.

RESOURCES

Vieillefond C. Programming the 80286 San Francisco: Sybex Corporation, 1987.

Jourdain R. Programmer's Problem Solver for the IBM PC, XT and AT New York: Brady Books, 1986.

Norton P. The New Peter Norton Programmer's Guide to the IBM PC and PS/2 Redmond: Microsoft Press, 1988.

Holzner S. PS/2-PC Assembly Language New York: Brady Books, 1989.

Waite M, Prata S. The Waite Group's New C Primer Plus Carmel: Howard W. Sams and Company, 1990.

Microsoft Corporation. CompuAdd MS-DOS Getting Started Austin: Microsoft Corporation, 1991.

Microsoft Corporation. User's Guide to Windows Austin: Microsoft Corporation, 1992.

20 --Dahle

WordPerfect Corporation. WordPerfect for Windows. Orem: WordPerfect Corporation, 1991.

Microsoft Corporation. Microsoft C Optimizing Compiler Austin: Microsoft Corporation, 1987.

Microsoft Corporation. CodeView and Utilities Austin: Microsoft Corporation, 1987.

Microsoft Corporation. Microsoft C Language Reference Austin: Microsoft Corporation, 1987.

Microsoft Corporation. Microsoft QuickC Compiler Austin: Microsoft Corporation, 1987.

Microsoft Corporation. Microsoft FORTRAN Reference Manual Austin: Microsoft Corporation, 1991.

FIGURES

1. This graph represents all the data collected in this experiment graphed in 3-dimensions. The x-axis represents the wavelength values from 480 to 650 nanometers, in 1-nanometer steps, the y-axis the absorbance values, and the z-axis the partial pressure of oxygen over time.

2. This figure shows the different components of the experimental system. The reaction chamber, oxygen concentration electrode, monitor and recorder, the spectrophotometric system and its control of the oxygen system with the TTL pulse, the gas-flow system to oxygenate the hemoglobin, and the system to keep the temperature in the reaction chamber constant.

3. This graph represents the fractional saturation of hemoglobin computed as a function of the partial pressure of oxygen in mm Hg. The fractional saturation of hemoglobin is computed using the following formula:

Fractional Saturation = %oxyHb / (%oxyHb + %deoxyHb).

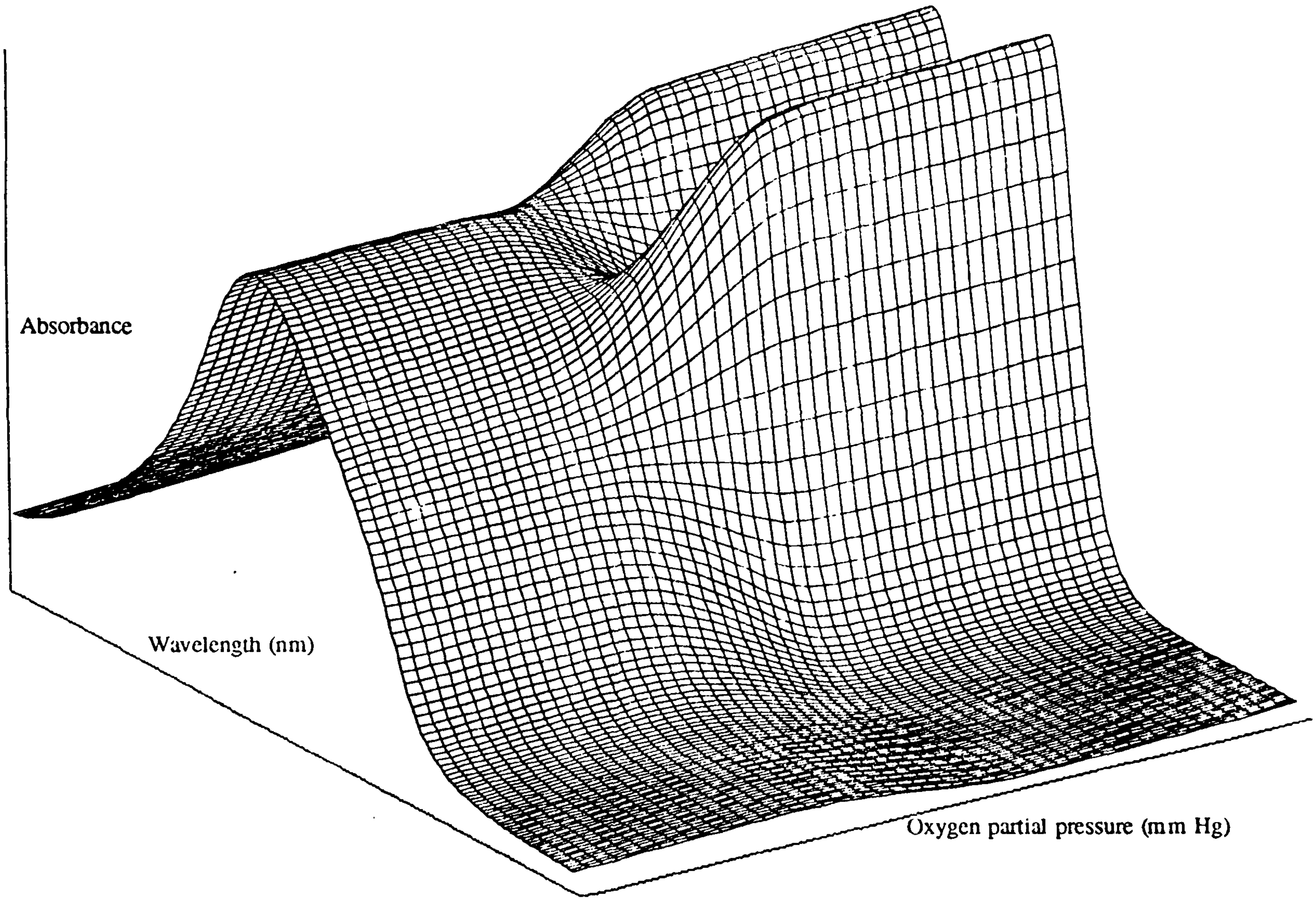


Figure 1

Rapid Scan OEC Apparatus

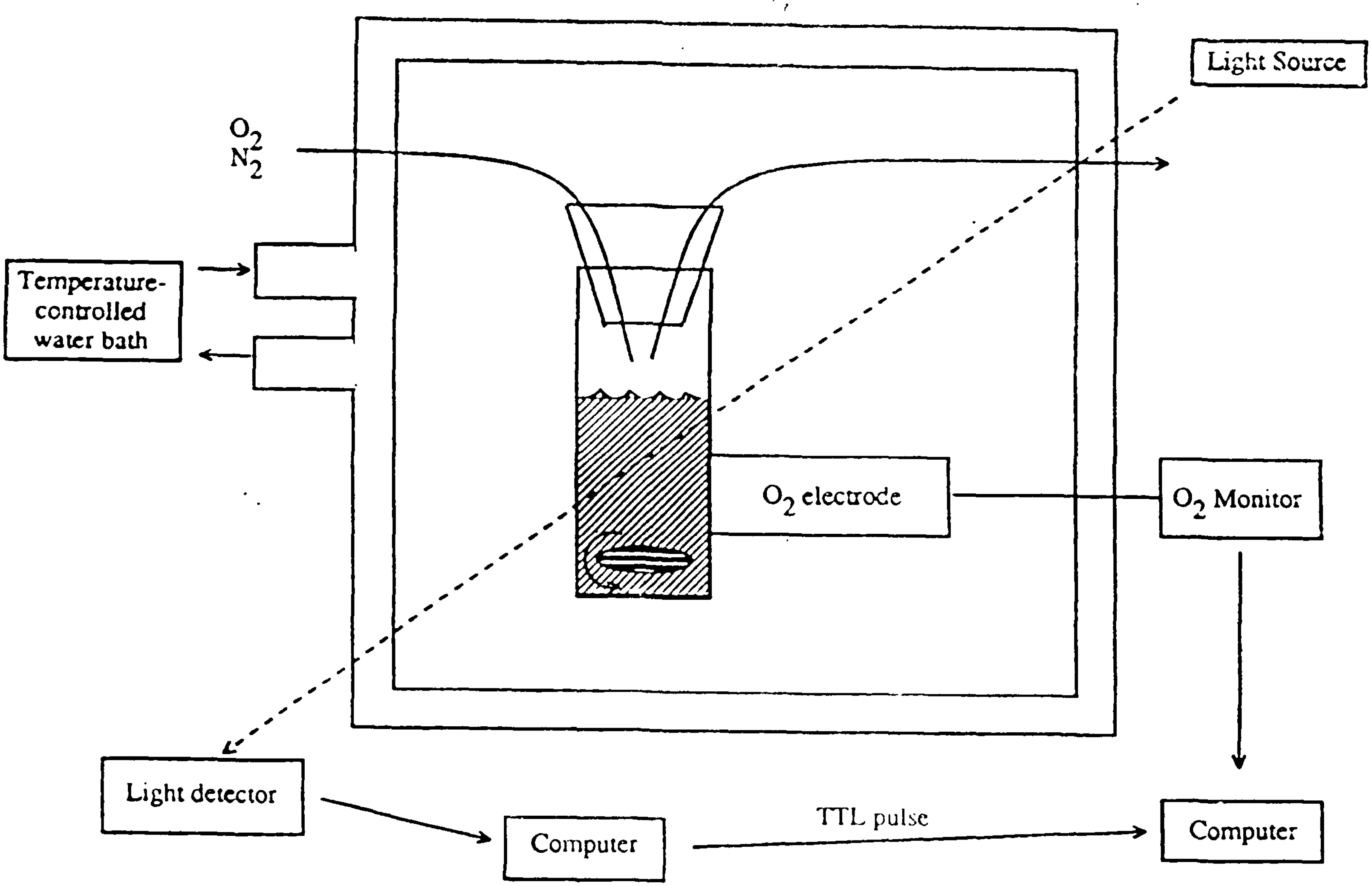


Figure 2

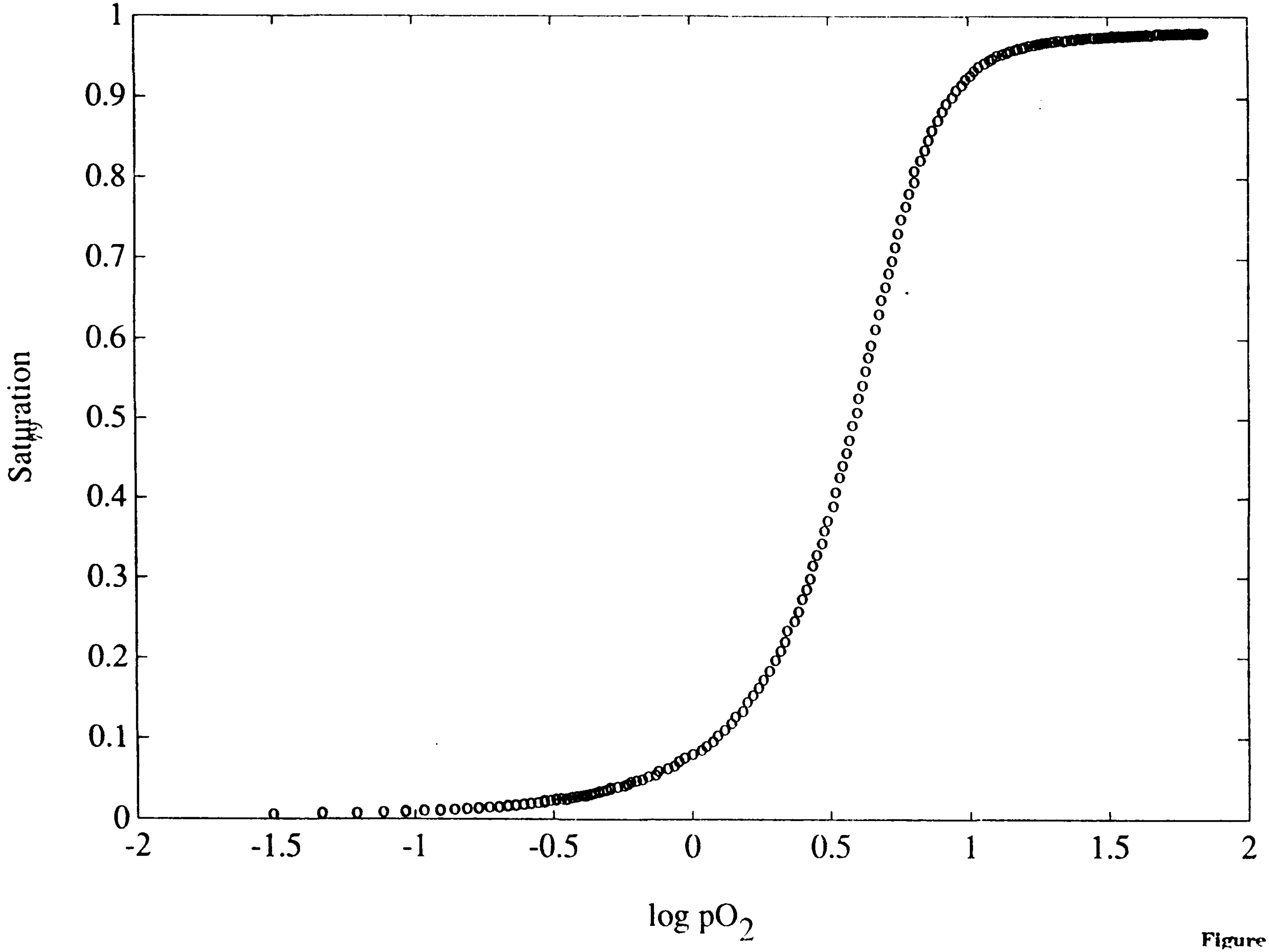


Figure 3

Appendix A

Version 1 of the oxygen voltage concentration recorder.

```
#include <stdio.h>
#include <stdlib.h>
#include <math.h>
#include <signal.h>
#include <ctype.h>
#include <dos.h>
#include <string.h>

extern void SH_CALL(char *,float *,unsigned short *);

#define ANACNT  8                /* Number of analog channels */
#define DIGCNT  12               /* Number of digital channels */
#define TRUE    1L
#define FALSE   NULL
unsigned short  digital[DIGCNT];         /* Digital channel array */
float           analog[ANACNT];          /* Analog channel array */
signed int      exitflag=FALSE;          /* exit flag */


/*
 * initial - sets up the hardware, reads calibration settings and set
 *         the channels
 */

int initialize(void)
{
        register int i;

        /* Read CALIB.DAT file and calibrate the analog inputs */
        SH_CALL("Fn",analog,digital);          /* Call A-D driver */

        if (digital[0]==0 && digital[2]==0)  {
                printf("\nERROR: Driver, ADRIVE.COM, not installed; ");
                printf(" or analog card not installed.\n");
                return(1);
        }

        if (digital[0]==0 && digital[2]!=0)  {
printf("\nERROR: No analog card selected. BRD SEL switch set to 0.\n");
                return(1);
        }

        if (digital[0]!=0 && digital[6]==0)  {
printf("\nERROR: CALIB.DAT file not correct or FIND.EXE was not run.\n");
                return(1);
        }

        if (digital[0] > digital[6])  {
                printf("\nERROR: Calibration numbers are not correct.\n");
                return(1);
        }

        if (digital[0]>ANACNT || digital[2]>DIGCNT)  {
                printf("\nERROR: Too many channels installed.\n");
                printf("Change size of ANACNT and/or DIGCNT in heading\n");
                return(1);
        }

        /* set number of analog input channels to use */
        digital[0]=1;                           /* use one analog channel */
        SH_CALL("N",analog,digital);

        /* Set analog channel resolution */
        digital[0]=16;                          /* 16 bit mode */
```

```
        /* Set up analog channel range and calibrate */
        digital[0]=2;                           /* 10 volts */
        SH_CALL("rc",analog,digital);

        /* Set up digital channel direction */
        for (i=0; i<DIGCNT; i++) digital[i]=0;  /* set for input */
        SH_CALL("S",analog,digital);

        /* Set delay time to zero */
        digital[0]=0;
        SH_CALL("d",analog,digital);                /* set delay value */

}


/*
 * Usage - prints out swithes and tells their usage
 */

void Usage(char **argv)
{
        printf("\nUsage: %s [switches]\r\n",argv[0]);
        printf("  -a[filename]  write O2 average to file\n");
        printf("  -d[filename]  write all data to file (.xxx added)\n");
        printf("  -h[number]    sampling rate in hertz (default=173)\n");
        printf("  -n[number]    number of sets to collect\n");
        printf("                  default: read until user interrupts\n");
        printf("  -t            print time taken to read oxygen voltages\n");
        printf("  -w            print all data to screen\n");
        printf("  -z[number]    scans per sample (default=8)\n\n");
}


/*
 * mkdfname - adds an extension to the oxygen voltage reading file
 *               based on the number of the SET that we are on
 *
 *  name=name of the file (without extension)
 *  dfnum=SET number we are on
 */

void mkdfname(char *name, int dfnum)
{
        register int i;
        char ext[4];

        sprintf(&ext[0],"%03d",dfnum);

        for (i=0; i<13; i++) {
                if (name[i]=='.') {
                        strcpy(&name[i+1],&ext[0]);
                        break;
                } else if (name[i]==0) {
                        name[i]='.';
                        strcpy(&name[i+1],&ext[0]);
                        break;
                }
        }
}


/*
 * chandler - function called when Control-C is pressed. Sets the exit
 *         flag to TRUE.
```

```
int chandler(void)
{
        signal(SIGINT, SIG_IGN);          /* disallow Ctr-C during handler */
        exitflag=TRUE;
        signal(SIGINT,chandler);          /* reattach handler to CTR-C */

        return 0;
}


/*
 * main - the program!!!
 */

int main(int argc, char **argv)
{
        register int i;           /* general purpose loop counter */
        int pdata=FALSE;          /* print all voltage readings to screen */
        int petime=FALSE;         /* print time taken to read oxygen voltages */
        int scansps=8;            /* scans per sample */
        int setlim=-1;            /* number ofsets */
        int dfon=FALSE;           /* write all voltage readings to a file */
        int hertz=173;            /* hertz */
        int samplecount;          /* number of voltage readings to make avr */
        int dfnum;                /* number of SETS of voltage readings taken */
        FILE *datafile=NULL,*avrfile=NULL;      /* file handles */
        char dfname[50],afname[50];     /* file names */
        float sum,avr,*buf;       /* variables for computing the avr V */
        struct dostime_t time1, time2;  /* variables used for elasped time */
        signed int s,h;

        /* set CTR-C handler so we can exit properly */
        if (signal(SIGINT,chandler)==(int(*) ())-1) {
                printf("ERROR: Unable to set CTR-C handler");
                return(1);
        }

        /* do hardware initialization and make sure driver is installed */
        if (initialize()) return(1);

        /* process switches */
        for (i=1; i<argc; i++) {
                if (!(argv[i][0]=='-')) {
                        printf("ERROR: Switch error\n");
                        Usage(argv);
                        goto end;
                } else {
                        switch(tolower(argv[i][1])) {
                                case 'a' :
                                        if (argv[i][2]==0)    /* default name */
                                                strcpy(&afname[0],"O2AVR.DAT");
                                        else    /* use user-defined name */
                                                strcpy(&afname[0],&argv[i][2]);
                                        avrfile=fopen(&afname[0],"w");
                                        if (!avrfile) {
                        printf("ERROR: Unable to open file %s\n",&afname[0]);
                                                goto end;
                                        }
                                        break;
                                case 'd' :
                                        if (argv[i][2]==0)    /* default name */
                                                strcpy(&dfname[0],"O2VDAT");
                                        else    /* use user-defined name */
                                                strcpy(&dfname[0],&argv[i][2]);
```

```
                                        break;
                                case 'h' :
                                        hertz=atoi(&argv[i][2]);
                                        break;
                                case 'n' :
                                        setlim=atoi(&argv[i][2]);
                                        break;
                                case 't' :
                                        petime=TRUE;
                                        break;
                                case 'w' :
                                        pdata=TRUE;
                                        break;
                                case 'z' :
                                        scansps=atoi(&argv[i][2]);
                                        break;
                                case '?' :
                                        Usage(argv);
                                        goto end;
                                default :
                        printf("ERROR: Unknown Switch '%c'\n",argv[i][1]);
                                        Usage(argv);
                                        goto end;
                        }
                }
        }

        /* compute number of readings to take */
        samplecount=scansps*0.2*hertz;

        /* allocate memory to read voltage data into */
        buf=(float *)malloc(samplecount*sizeof(float));
        if (!buf) {
                printf("ERROR: Not enough memory\n");
                goto end;
        }

        /* print out information for user */
        printf("Hertz: %d\n",hertz);
        printf("Scans Per Sample: %d\n",scansps);
        printf("Number of Voltage Readings to take: %d\n",samplecount);
        if (avrfile) printf("Writing Averages to file: %s\n",&afname[0]);
        if (dfon) printf("Writing Voltage Readings to files: %s.xxx\n",&dfname[0
]);
        if (setlim==-1) {
                printf("\nHit Control-C to Stop.\n");
        } else {
                printf("Number of Sets to read: %d\n",setlim);
                printf("Hit Control-C to Abort.\n\n");
        }

        printf("Waiting for TTL Signal...\n");
        for (dfnum=1; (setlim==-1)?(1):(dfnum<=setlim); dfnum++) {
                if (exitflag) { printf("Terminating...\n"); goto end; }
                if (dfon) {
                        mkdfname(&dfname[0],dfnum);
                        datafile=fopen(&dfname[0],"w");
                        if (!datafile) {
                printf("ERROR: Unable to open file %s\n",&dfname[0]);
                                goto end;
                        }
                }

                do {    /* wait for TTL signal */
                        if (exitflag) { printf("Terminating...\n"); goto end; }
```

```
            } while (digital[0]==1);
            if (exitflag) { printf("Terminating...\n"); goto end; }

            /* collect O2 voltages */
            digital[0]=samplecount;
            digital[1]=0;
            if (petime) {
                    _dos_gettime(&time1);
                    SH_CALL("M",buf,digital);
                    _dos_gettime(&time2);
                    s=(int)time2.second-time1.second;
                    h=(int)time2.hsecond-time1.hsecond;
                    if (h<0) { h=100+h; s--; }
                    printf("Elasped Time = %d.%d seconds\n",s,h);
            } else {
                    SH_CALL("M",buf,digital);
            }
            if (exitflag) { printf("Terminating...\n"); goto end; }

            /* compute average average oxygen voltage */
            if (pdata) printf("Oxygen Voltage Data: #%d\n",dfnum);
            for (i=0,sum=(float)0; i<samplecount; i++) {
                   if (pdata) printf("%20e",buf[i]);
                   if (datafile) fprintf(datafile,"%20e\r\n",buf[i]);
                   sum=sum+buf[i];
            }
            avr=sum/samplecount;
    printf("Average Oxygen Voltage for %04d readings (%04d): %20e\n"
                                              ,samplecount,dfnum,avr);
            if (avrfile) fprintf(avrfile,"%20e\r\n",avr);

            /* close data file for oxygen voltage readings */
            if (datafile) {
                    fclose(datafile);
                    datafile=NULL;
            }
    }

  end :
    if (buf) free(buf);
    if (datafile) fclose(datafile);
    if (avrfile) fclose(avrfile);

    return(0);
}

/* end of file - 'o2volt.c' */
```

Appendix B

Version 2 of the oxygen voltage concentration recorder.

```
## --- Program: o2volt
## --- File: o2volt.lmk
## --- Author: David Dahle (summer apprentice)
## --- Date: July-August 1992
## --- Purpose: compile/assemble o2volt program
## --- Letterman Army Institute of Research
## --- Persidio of San Francisco, CA 94129-6800
##


BASE=o2volt
OBJ=obj
SOURCE=source
LST=listings
ASMOPT=/ml /z /n /c /l /Ik:\o2volt\source
COPT=/AS /FPi87 /c /Fo$(OBJ)\fmath.obj /Fa$(OBJ)\fmath.asm /W3 /Gs \
                                                /Fs$(LST)\fmath.lst
FILES=$(OBJ)\main.obj $(OBJ)\hard.obj $(OBJ)\util.obj $(OBJ)\fmath.obj \
        $(OBJ)\help.obj


$(BASE).exe: $(FILES)
        link /NOI $(FILES),$(BASE).exe ;

$(OBJ)\fmath.obj: $(SOURCE)\fmath.c
        cl $(COPT) $(SOURCE)\fmath.c

$(OBJ)\main.obj: $(SOURCE)\main.asm $(SOURCE)\header.i
        tasm $(ASMOPT) $(SOURCE)\main.asm,$(OBJ)\main.obj,$(LST)\main.lst

$(OBJ)\hard.obj: $(SOURCE)\hard,asm $(SOURCE)\header.i
        tasm $(ASMOPT) $(SOURCE)\hard.asm,$(OBJ)\hard.obj,$(LST)\hard.lst

$(OBJ)\util.obj: $(SOURCE)\util.asm $(SOURCE)\header.i
        tasm $(ASMOPT) $(SOURCE)\util.asm,$(OBJ)\util.obj,$(LST)\util.lst

$(OBJ)\help.obj: $(SOURCE)\help.asm $(SOURCE)\header.i
        tasm $(ASMOPT) $(SOURCE)\help.asm,$(OBJ)\help.obj,$(LST)\help.lst

$(SOURCE)\header.i:


## end of file 'o2volt.lmk'
```

```
;
; --- Program: o2volt
; --- File: header.i
; --- Author: David Dahle (summer apprentice)
; --- Date: July-August 1992
; --- Purpose: global program definitions
; --- Letterman Army Institute of Research
; --- Persidio of San Francisco, CA 94129-6800
;
;**********************************************************************


callsys macro    interrupt,service
        mov      ah,service
        int      interrupt
        endm


FNAME_LEN       equ     127
NUMBUF_LEN      equ     127
DACQ_ENTRIES    equ     3000
AVRFL_DWIDTH    equ     23
DATFL_DWIDTH    equ     25
MIDDLE_LINE     equ     13
DISPLAY_PAGE    equ     1h
DISPLAY_HEIGHT  equ     25
DISPLAY_WIDTH   equ     80

DISPLAY_NORMAL  equ     00010111b
DISPLAY_HILIGHT equ     00101111b

; end of file 'header.i'
```

```
;.........................................................**********************************
;
; --- Program: o2volt
; --- File: help.asm
; --- Author: David Dahle (summer apprentice)
; --- Date: July-August 1992
; --- Purpose: help text and functions for oxygen voltage recorder
; --- Letterman Army Institute of Research
; --- Persidio of San Francisco, CA 94129-6800
;
;*******************************************************************

        dosseg
        locals  __
        include header.i

        .model  small

        .data

;---------------12345678901234567890123456789O123-------------
begin   db      'The Begin function is used to ',0
        db      'start data acquisition.  Once',0
        db      'started, acquisition may be',0
        db      'terminated by pressing any key.',0
        db      0
instant db      'The Instant function displays',0
        db      'the current analog voltage level',0
        db      'and the current state of the',0
        db      'TTL signal.  This function can',
        db      'be terminated by pressing any',0
        db      'key.',0
        db      0
load    db      'The Load function allows a',0
        db      'config file with a name other',0
        db      'than the default name to be',0
        db      'loaded.  A string input field',0
        db      'will appear below the command',0
        db      'list where the name can be',0
        db      'entered.  ESCAPE aborts',0
        db      0
save    db      'The Save function allows a',0
        db      'config file to be saved to a',0
        db      'file with a name other than the',0
        db      'the default name.  A string',0
        db      'will appear below the command',0
        db      'bar where the new name can be',0
        db      'entered.  ESCAPE aborts',0
        db      0
exit    db      'The Exit function writes',0
        db      'the configuration settings',0
        db      'to the default config file',0
        db      'and then exits.',0
        db      0
avrfn   db      'The Average File field',0
        db      'specifies the name of the',0
        db      'file in which the averages',0
        db      'from each set will be written.',0
        db      'If no name is given, then no',0
        db      'file will be created.',0
        db      0
datfn   db      'The Data Files field',0
        db      'specifies the names of files',0
```

```
        db      'in which each readings from',0
        db      'each set will be written.',0
        db      'If no name is given, then no',0
        db      'files will be created.',0
        db      0
expnum  db      'The Experiment Number field',0
        db      'specifies the number of the',0
        db      'current experiment.  If the',0
        db      'Automatic File Names option',0
        db      'is turned off, this number is',0
        db      'ignored.',0
        db      0
autofns db      'The Automatic File Names',0
        db      'field lets you turn this',0
        db      'feature on or off. If on, the',0
        db      'program will create file names',0
        db      'based on the current date and',0
        db      'the experiment number.',0
        db      0
prtt    db      'The Print Time field lets you',0
        db      'specify if you want the elasped',0
        db      'time that the TTL pulse was',0
        db      'active and the data rate in',0
        db      'hertz to be computed.',0
        db      0
range   db      'The Analog Input Range field',0
        db      'lets you specify the projected',0
        db      'range of the analog voltage',0
        db      'signal. These are listed on',0
        db      'page 185 in the manual and are',0
        db      'used by the "r" command.',0
        db      0
resol   db      'The Analog Input Resolution',0
        db      'field lets you specify the',0
        db      'resolution of the readings.',0
        db      'These are listed on page 148',0
        db      'in the manual and are used by',0
        db      'the "a" command.',0
        db      0


        ...le
  ... that appear in file 'util.asm'
        extrn   printStringLimit:near


        ... displayHelpText
        ... si=pointer to set of null-teriminated null-terminated strings
```

```
        je      short __continue
__strloop:
        mov     al,[si]
        inc     si
        cmp     al,0
        jne     short __strloop
__continue:
        inc     dh                          ; inc line number
        loop    short __loop
__endOfText:
        ret



;
; --- Procedure: displayCmdHelp
; --- Input: al=[command]
; --- Output:
;

        public  displayCmdHelp
displayCmdHelp:
        cmp     al,0
        jne     __not1
        call    displayBeginHelp
        jmp     __end
__not1: cmp     al,1
        jne     __not2
        call    displayInstantHelp
        jmp     __end
__not2: cmp     al,2
        jne     __not3
        call    displayLoadHelp
        jmp     __end
__not3: cmp     al,3
        jne     __not4
        call    displaySaveHelp
        jmp     __end
__not4: cmp     al,4
        jne     __not5
        call    displayExitHelp
        jmp     __end
__not5:
__end:  ret



        public  displayBeginHelp
displayBeginHelp:
        lea     si,begin
        call    displayHelpText
        ret
        public  displayInstantHelp
displayInstantHelp:
        lea     si,instant
        call    displayHelpText
        ret
        public  displayLoadHelp
displayLoadHelp:
        lea     si,load
        call    displayHelpText
        ret
        public  displaySaveHelp
displaySaveHelp:
        lea     si,save
        call    displayHelpText
```

```
        public  displayExitHelp
displayExitHelp:
        lea     si,exit
        call    displayHelpText
        ret
        public  displayAvrfnHelp
displayAvrfnHelp:
        lea     si,avrfn
        call    displayHelpText
        ret
        public  displayDatfnHelp
displayDatfnHelp:
        lea     si,datfn
        call    displayHelpText
        ret
        public  displayExpnumHelp
displayExpnumHelp:
        lea     si,expnum
        call    displayHelpText
        ret
        public  displayAutofnsHelp
displayAutofnsHelp:
        lea     si,autofns
        call    displayHelpText
        ret
        public  displayPrttHelp
displayPrttHelp:
        lea     si,prtt
        call    displayHelpText
        ret
        public  displayRangeHelp
displayRangeHelp:
        lea     si,range
        call    displayHelpText
        ret
        public  displayResolHelp
displayResolHelp:
        lea     si,resol
        call    displayHelpText
        ret


        end
; end of file 'help.asm'
```

```
;
; --- Program: o2volt
; --- File: main.asm
; --- Author: David Dahle (summer apprentice)
; --- Date: July-August 1992
; --- Purpose: main program module for oxygen voltage reader
; --- Letterman Army Institute of Research
; --- Persidio of San Francisco, CA 94129-6800
;
;***********************************************************************

        dosseg
        locals
        include header.i


        .model  small


        .data
; display text
ptitle  db      ' Data Acquisition                                          Oxygen
Voltage Recorder ',0
pline   db      '³',0
pbtop   db      'ÕÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍProgramÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍ
ÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍ¸',0
pbmid   db      'ÆÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍDialogÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍ
ÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍµ',0
pbbot   db      'ÔÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍ
ÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍ¾',0
pctop   db      'ÕÍÍÍÍÍÍÍÍÍÍÍÍÍÍHelpÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍ¸',0
pcbot   db      'ÔÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍ¾',0
avrfl_t db      'Average File: ',0
datfl_t db      'Data Files: ',0
autnu_t db      'Experiment Number: ',0
autfn_t db      'Automatic File Naming: ',0
etime_t db      'Print Elasped Time: ',0
range_t db      'Analog Input Range: ',0
resol_t db      'Analog Input Resolution: ',0
etime_1 db      'ON ',0
etime_2 db      'OFF',0
range_0 db      '50 mV     ',0          ; these must be spaces here!!
range_1 db      '500 mV    ',0
range_2 db      '10 V      ',0
range_3 db      '+/-25 mV  ',0
range_4 db      '+/-250 mV ',0
range_5 db      '+/-5 V    ',0
range_6 db      '2 mA      ',0
range_7 db      '20 mA     ',0
range_8 db      '+/-1 mA   ',0
range_9 db      '+/-10 mA  ',0
rang_10 db      '+/-50 mA  ',0
rang_16 db      'AR /w uniV',0
rang_17 db      'AR /w biV ',0
rang_18 db      'AR /w uniC',0
rang_19 db      'AR /w biC ',0
resol_1 db      'low noise',0
resol_2 db      '16 bits  ',0
resol_3 db      '15 bits  ',0
resol_4 db      '14 bits  ',0
resol_5 db      '13 bits  ',0
resol_6 db      '12 bits  ',0
numcom  db      4                       ; number of command items -1
comm_0  db      ' Begin ',0
comm_1  db      ' Instant ',0
```

```
comm_3  db      ' Save ',0
comm_4  db      ' Exit ',0

; startup message
startup db      'Data Acquisition Program - Oxygen Voltage Recorder',0
        db      'Strawberry Tree Incorporated  Analog-to-Digital Converter',0
        db      'Letterman Army Institute of Research',0
        db      'Persidio of San Francisco, CA 94129-6800',0
blank1  db      ' ',0
        db      'Author: David Dahle',0
        db      'Version: 2.10  Assembled: ',??date,0
        db      0
hard_er db      ' Press any key to exit...',0
hard_sc db      'Setup completed successfully.',0

; program variables
curdate dw      2 DUP (0)
avrfl   db      FNAME_LEN DUP (0)
datfl   db      FNAME_LEN DUP (0)
numbuf  db      NUMBUF_LEN DUP (0)
etime   dw      1                   ; 1=ON, 2=OFF
range   dw      5                   ; range of analog input
resol   dw      2                   ; resolution of analog input
autofn  dw      2                   ; 1=ON  2=OFF
autonum dw      1                   ; number for automatic increment
config_defaultName      db          'o2volt.cnf',0

; program status
mode    db      0                   ; 0=com,1=config
command db      0                   ; 0=begin,1=instant,2=hardware info,3=exit
com_sav db      0                   ; saved copy of command
config  db      1                   ; config line 1-7


        global  resol:byte,avrfl:byte,datfl:byte,range:word,etime:byte
        global  startup:byte,curdate:word,autonum:word,blank1:byte
        global  autofn:word


        .code
; labels that appear in 'util.asm'
        extrn   printTextToScreen:near
        extrn   setUpStringGadget:near,setDisplayAndCursorPos:near
        extrn   removeStringGadget:near,addStringGadgetChar:near
        extrn   delStringGadgetChar:near,backStringGadgetChar:near
        extrn   leftStringGadgetChar:near,rightStringGadgetChar:near
        extrn   printCursorString:near,printString:near
        extrn   printCursorStringAttrib:near,printStringAttrib:near
        extrn   printCursorStringLimit:near,printStringLimit:near
        extrn   convertItoA:near,convertLtoA:near,convertAtoI:near
        extrn   loadConfigurationFile:near,saveConfigurationFile:near
        extrn   getConfigFileName:near,clearKeyBoardBuffer:near

; labels that appear in 'hard.asm'
        extrn   setUpHardware:near,instant_Readings:near
        extrn   initializeHardware:near,beginDataAcquisition:near

; labels that apprear in 'help.asm'
        extrn   displayResolHelp:near,displayRangeHelp:near
        extrn   displayPrttHelp:near,displayAutofnsHelp:near
        extrn   displayExpnumHelp:near,displayDatfnHelp:near
        extrn   displayAvrfnHelp:near,displayCmdHelp:near

;
; --- Procedure   main
```

```
; --- Output:
; --- Preserves:
;
;       called by C-Language startup code
;

        public  _main
_main:
; get current date
        callsys 21h,2ah                   ; get date
        mov     [curdate],cx              ; year
        mov     [curdate+2],dx            ; month,day

; setup screen display
        call    initDisplay               ; color, borders
        call    print_allConfig           ; configuration
        call    print_startupMessage      ; dialog window

; setup hardware
        call    setUpHardware
        cmp     ax,0
        je      short __noHardwareErrors

; error with A/D board, exit program
        mov     si,dx
        call    printTextToScreen         ; print error text
        call    print_cmdLineBusy
        callsys 16h,0
        jmp     short __exitProgram

__noHardwareErrors:
        call    initializeHardware
        call    print_cmdLine
        mov     si,offset hard_sc
        call    printTextToScreen

__main:
        mov     al,[command]
        call    displayCmdHelp
        lea     dx,config_defaultName
        call    loadConfigurationFile
        call    mainLoop
        lea     dx,config_defaultName
        call    saveConfigurationFile

__exitProgram:
        call    clearKeyBoardBuffer
        call    resetDisplay
        mov     ax,0              ; set a return code
        ret


;
; --- Procedure: print_startupMessage
; --- Input: none
; --- Output: none
; --- Preserves:
;

print_startupMessage:
        mov     si,OFFSET startup
_startMessage:
        push    si
        call    printTextToScreen
```

```
__loop2:
        mov     al,[si]
        inc     si
        cmp     al,0
        jne     __loop2
        mov     al,[si]                         ; null-string=end of startupText
        cmp     al,0
        je      short __startMessageEnd
        jmp     __startMessage
__startMessageEnd:
        ret



;
; --- Procedure: mainLoop
; --- Input: none
; --- Output: none
;

mainLoop:
        callsys 16h,0           ; wait for next character

        cmp     al,0            ; check for extended characterr
        je      short __extended
        cmp     al,13           ; check for return key
        jne     short __notReturn
        cmp     [mode],0
        jne     short __notCommRet
        mov     al,[command]
        cmp     al,0
        jne     short __notComm0
        call    beginDataAcquisition
        jmp     mainLoop
__notComm0:
        cmp     al,1
        jne     short __notComm1
        call    instant_Readings
        jmp     mainLoop
__notComm1:
        cmp     al,2
        jne     short __notComm2
        call    getConfigFileName
        cmp     ax,0
        je      short __comm2Exit
        call    loadConfigurationFile
__comm2Exit:
        jmp     mainLoop
__notComm2:
        cmp     al,3
        jne     short __notComm3
        call    getConfigFileName
        cmp     ax,0
        je      short __comm3Exit
        call    saveConfigurationFile
__comm3Exit:
        jmp     mainLoop
__notComm3:
        jmp     short __exit
__notCommRet:
        call    handleConfigGadgetReturn
        jmp     mainLoop

__notReturn:
        cmp     al,8                            ; check for backspace
```

```
        cmp     [mode],1
        jne     short __notBackConfigGadget
        cmp     [config],4
        jg      short __notBackStringGadget
        call    backStringGadgetChar
__notBackStringGadget:
__notBackConfigGadget:
        jmp     mainLoop

__notBackspace:
        cmp     al,32                   ; a printable character?
        jl      short __notPrintChar
        cmp     al,126
        jg      short __notPrintChar
        call    addStringGadgetChar
        jmp     mainLoop

__notPrintChar:
        jmp     mainLoop

__exit: ret

__extended:
        cmp     ah,75
        jne     short __notLeft         ; check for Cursor Left
        cmp     byte ptr [mode],0
        jne     short __notLeftMode0
        mov     al,[command]
        cmp     al,0
        jne     short __notUnder
        mov     al,[numcom]
        jmp     short __under
__notUnder:
        dec     al
__under:
        mov     [command],al
        call    print_cmdLine
        mov     al,[command]
        call    displayCmdHelp
        jmp     mainLoop
__notLeftMode0:
        cmp     [config],4
        jg      short __notLeftStringGadget
        call    leftStringGadgetChar
__notLeftStringGadget:
        jmp     mainLoop

__notLeft:
        cmp     ah,77
        jne     short __notRight        ; check for Cursor Right
        cmp     byte ptr [mode],0
        jne     short __notRightMode0
        mov     al,[command]
        inc     al
        cmp     al,[numcom]
        jle     short __notOver
        mov     al,0
__notOver:
        mov     [command],al
        call    print_cmdLine
        mov     al,[command]
        call    displayCmdHelp
        jmp     mainLoop
__notRightMode0
        cmp     [config],4
```

```
        call    rightStringGadgetChar
__notRightStringGadget:
        jmp     mainLoop

__notRight:
        cmp     ah,72
        jne     short __notUp              ; check for Cursor Up
        cmp     [mode],0
        jne     short __notCommUp
        mov     al,[command]               ; remove hilight display
        mov     [com_sav],al
        mov     [command],-1
        mov     [mode],1
        mov     [config],7                 ; bottom editing gadget
        call    print_cmdLine
        call    handleConfigGadgetSetUp
        jmp     mainLoop
__notCommUp:
        cmp     [config],1
        jne     short __notAtTheTop
        call    handleConfigGadgetRemoval
        mov     byte ptr [mode],0
        mov     al,[com_sav]
        mov     [command],al
        call    print_cmdLine
        mov     al,[command]
        call    displayCmdHelp
        jmp     mainLoop
__notAtTheTop:
        call    handleConfigGadgetRemoval
        dec     [config]
        call    handleConfigGadgetSetUp
        jmp     mainLoop

__notUp:
        cmp     ah,80
        jne     short __notDown            ; check for Cursor Down
        cmp     [mode],0
        jne     short __notCommDown
        mov     al,[command]               ; remove hilight display
        mov     [com_sav],al
        mov     [command],-1
        mov     [mode],1
        mov     [config],1                 ; bottom editing gadget
        call    print_cmdLine
        call    handleConfigGadgetSetUp
        jmp     mainLoop
__notCommDown:
        cmp     [config],7
        jne     short __notAtTheBottom
        call    handleConfigGadgetRemoval
        mov     [mode],0
        mov     al,[com_sav]
        mov     [command],al
        call    print_cmdLine
        mov     al,[command]
        call    displayCmdHelp
        jmp     mainLoop
__notAtTheBottom:
        call    handleConfigGadgetRemoval
        inc     [config]
        call    handleConfigGadgetSetUp
        jmp     mainLoop

__notDown:
```

```
        jne      short __notDelete
        cmp      [mode],1
        jne      short __notDelConfig
        cmp      [config],4
        jg       short __notDelStringGadget
        call     delStringGadgetChar
__notDelStringGadget:
__notDelConfig:
        jmp      mainLoop

__notDelete:
        jmp      mainLoop



;
; --- Procedure: handleConfigGadgetSetUp
; --- Input: none
; --- Output: none
; --- Preserves:
;

handleConfigGadgetSetUp:
        mov      al,[config]
        cmp      al,1
        jne      short __notConfig1
        call     displayAvrfnHelp
        mov      al,0                         ; al=0 string gadget
        mov      bx,offset avrfl
        mov      cl,FNAME_LEN
        mov      ch,AVRFL_DWIDTH
        mov      dl,17
        mov      dh,3
        call     setUpStringGadget
        jmp      __exit

__notConfig1:
        cmp      al,2
        jne      short __notConfig2
        call     displayDatfnHelp
        mov      al,0                         ; al=0 string gadget
        mov      bx,offset datfl
        mov      cl,FNAME_LEN
        mov      ch,DATFL_DWIDTH
        mov      dl,15
        mov      dh,4
        call     setUpStringGadget
        jmp      __exit

__notConfig2:
        cmp      al,3
        jne      short __notConfig3
        call     displayExpnumHelp
        mov      ax,[autonum]
        call     convertItoA                  ; return ds:bx
        mov      si,offset nedbuf
__config3Loop:
        mov      al,[bx]
        inc      bx
        mov      [si],al
        inc      si
        cmp      al,0
        jne      __config3Loop
        mov      al,1                         ; al=1 number gadget
        mov      bx,offset nedbuf
```

```
        mov     ch,5
        mov     dl,22
        mov     dh,5
        call    setUpStringGadget
        jmp     __exit

__notConfig3:
        cmp     al,4
        jne     short __notConfig4
        call    displayAutofnsHelp
        mov     bl,DISPLAY_HILIGHT
        call    print_autofn
        jmp     __exit

__notConfig4:
        cmp     al,5
        jne     short __notConfig5
        call    displayPrttHelp
        mov     bl,DISPLAY_HILIGHT
        call    print_etime
        jmp     __exit

__notConfig5:
        cmp     al,6
        jne     short __notConfig6
        call    displayRangeHelp
        mov     bl,DISPLAY_HILIGHT
        call    print_range
        jmp     __exit

__notConfig6:
        cmp     al,7
        jne     __notConfig7
        call    displayResolHelp
        mov     bl,DISPLAY_HILIGHT
        call    print_resol

__notConfig7:
__exit:
        ret


;
; --- Procedure: handleConfigGadgetRemoval
; --- Input: none
; --- Output: none
;

handleConfigGadgetRemoval:
        mov     al,[config]
        cmp     al,1
        jne     short __notConfig1
        call    removeStringGadget
        jmp     short __exit

__notConfig1:
        cmp     al,2
        jne     short __notConfig2
        call    removeStringGadget
        jmp     short __exit

__notConfig2:
        cmp     al,3
        jne     short __notConfig3
```

```
        mov     si,offset newbuf
        call    convertAtoI
        mov     [autonum],ax
        call    print_autonum
        jmp     short __exit

__notConfig3:
        cmp     al,4
        jne     short __notConfig4
        mov     bl,DISPLAY_NORMAL
        call    print_autofn
        jmp     short __exit

__notConfig4:
        cmp     al,5
        jne     short __notConfig5
        mov     bl,DISPLAY_NORMAL
        call    print_etime
        jmp     short __exit

__notConfig5:
        cmp     al,6
        jne     short __notConfig6
        mov     bl,DISPLAY_NORMAL
        call    print_range
        jmp     short __exit

__notConfig6:
        cmp     al,7
        jne     short __notConfig7
        mov     bl,DISPLAY_NORMAL
        call    print_resol
;       jmp     short __exit

__notConfig7:
__exit:
        ret



;
; --- Procedure: handleConfigGadgetReturn
; --- Input: none
; --- Output: none
;

handleConfigGadgetReturn:
        mov     al,[config]

__notConfig3:
        cmp     al,4
        jne     short __notConfig4
        mov     bl,DISPLAY_NORMAL
        call    print_autofn
        mov     ax,[autofn]
        cmp     ax,2
        jne     short __notAutofn1
        mov     ax,1
        jmp     short __notAutofn2
__notAutofn1:
        inc     ax
__notAutofn2:
        mov     [autofn],ax
        mov     bl,DISPLAY_HILIGHT
        call    print_autofn
```

```
__notConfig4:
        cmp     al,5
        jne     short __notConfig5
        mov     bl,DISPLAY_NORMAL
        call    print_etime
        mov     ax,[etime]
        cmp     ax,2
        jne     short __notEtime1
        mov     ax,1
        jmp     short __notEtime2
__notEtime1:
        inc     ax
__notEtime2:
        mov     [etime],ax
        mov     bl,DISPLAY_HILIGHT
        call    print_etime
        jmp     short __exit


__notConfig5:
        cmp     al,6
        jne     short __notConfig6
        mov     bl,DISPLAY_NORMAL
        call    print_range
        mov     ax,[range]
        cmp     ax,10
        jg      short __notC6Less16
        cmp     ax,10
        jne     short __notC6LoopAround
        mov     ax,16
        jmp     short __config6End

__notC6Less16:
        cmp     ax,19
        jne     short __notC6LoopAround
        mov     ax,0
        jmp     short __config6End

__notC6LoopAround:
        inc     ax

__config6End:
        mov     [range],ax
        mov     bl,DISPLAY_HILIGHT
        call    print_range
        jmp     short __exit

__notConfig6:
        cmp     al,7
        jne     short __notConfig7
        mov     bl,DISPLAY_NORMAL
        call    print_resol
        mov     ax,[resol]
        cmp     ax,6
        jne     short __notResol1
        mov     ax,1
        jmp     short __notResol2
__notResol1:
        inc     ax
__notResol2:
        mov     [resol],ax
        mov     bl,DISPLAY_HILIGHT
        call    print_resol
        jmp     short __exit
```

```
__notConfig7:
__exit:
        ret


;
; --- Procedure: print_cmdLineBusy
; --- Input:
; --- Outpu:
; --- Preserves:
;

        public  print_cmdLineBusy
print_cmdLineBusy:
        mov     bh,DISPLAY_PAGE
        mov     dl,3
        mov     dh,11
        callsys 10h,2
        mov     ah,9
        mov     al,' '
        mov     bh,DISPLAY_PAGE
        mov     bl,DISPLAY_NORMAL
        mov     cx,40
        callsys 10h,9           ; BIOS write character
        lea     si,hard_er
        mov     dl,3
        mov     dh,11
        call    printString
        ret


;
; --- Procedure: print_cmdLine
; --- Input: none
; --- Output: none
; --- Preserves: ds,es,si,di
;

        public  print_cmdLine
print_cmdLine:
        mov     bh,DISPLAY_PAGE
        mov     dl,3
        mov     dh,11
        callsys 10h,2           ; set cursor position
        mov     si,offset comm_0
        mov     cx,0
__loop: push    cx
        cmp     [command],cl
        je      short __jmp0
        mov     bl,DISPLAY_NORMAL
        jmp     short __jmp1
__jmp0: mov     bl,DISPLAY_HILIGHT
__jmp1: call    printCursorStringAttr
        pop     cx
        inc     cx
        cmp     [numcom],cl
        jge     __loop
        ret


;
; --- Procedure: print_all ...
```

```
; --- Output: none
; --- Preserves: ...?
;
        public  print_datfl,print_avrfl,print_autonum,print_allConfig
print_allConfig:
        call    print_avrfl
        call    print_datfl
        call    print_autonum
        mov     bl,DISPLAY_NORMAL
        call    print_autofn
        mov     bl,DISPLAY_NORMAL
        call    print_etime
        mov     bl,DISPLAY_NORMAL
        call    print_range
        mov     bl,DISPLAY_NORMAL
        call    print_resol
        ret
print_avrfl:
        mov     si,offset avrfl_t
        mov     dh,3
        mov     dl,3
        call    printString
        mov     si,offset avrfl
        mov     cl,AVRFL_DWIDTH
        call    printCursorStringLimit
        ret
print_datfl:
        mov     si,offset datfl_t
        mov     dh,4
        mov     dl,3
        call    printString
        mov     si,offset datfl
        mov     cl,DATFL_DWIDTH
        call    printCursorStringLimit
        ret
print_autonum:
        mov     si,offset autnu_t
        mov     dh,5
        mov     dl,3
        call    printString
        mov     ax,[autonum]
        call    convertItoA
        mov     si,bx
        mov     cl,4                    ; max length of string
        call    printCursorStringLimit
        ret
print_autofn:
        mov     si,offset autfn_t
        mov     dh,6
        mov     dl,3
        call    printString
        mov     ax,[autofn]
        cmp     al,1
        jne     short __jmp1
        mov     si,offset etime_1
        jmp     short __jmp2
__jmp1: mov     si,offset etime_2
__jmp2: call    printCursorStringAttrib
        ret
print_etime:
        mov     si,offset etime_t
        mov     dh,7
        mov     dl,3
        call    printString
```

```
        cmp     al,1
        jne     short __jmp1
        mov     si,offset etime_1
        jmp     short __jmp2
__jmp1: mov     si,offset etime_2
__jmp2: call    printCursorStringAttrib
        ret
print_range:
        mov     si,offset range_t
        mov     dh,8
        mov     dl,3
        call    printString
        mov     ax,[range]
        cmp     ax,10
        jle     __notOver
        sub     ax,5
__notOver:
        mov     cl,11
        mul     cl
        lea     si,range_0
        add     si,ax
        call    printCursorStringAttrib
        ret
print_resol:
        mov     si,offset resol_t
        mov     dh,9
        mov     dl,3
        call    printString
        mov     ax,[resol]
        cmp     al,1
        jne     short __jmp1
        mov     si,offset resol_1
        jmp     short __jmpf
__jmp1: cmp     al,2
        jne     short __jmp2
        mov     si,offset resol_2
        jmp     short __jmpf
__jmp2: cmp     al,3
        jne     short __jmp3
        mov     si,offset resol_3
        jmp     short __jmpf
__jmp3: cmp     al,4
        jne     short __jmp4
        mov     si,offset resol_4
        jmp     short __jmpf
__jmp4: cmp     al,5
        jne     short __jmp5
        mov     si,offset resol_5
        jmp     short __jmpf
__jmp5: mov     si,offset resol_6
__jmpf: call    printCursorStringAttrib
        ret


;
; --- Procedure: initDisplay
; --- Input: none
; --- Output: none
; --- Preserves: ds,es ...?
;

initDisplay:
; make page the current display page
        mov     al,DISPLAY_PAGE
```

```
; set screen colors
        mov     bh,DISPLAY_PAGE
        mov     dx,0
        callsys 10h,2               ; Set Cursor Position (BIOS)
        mov     ah,9
        mov     al,' '
        mov     bh,DISPLAY_PAGE
        mov     bl,DISPLAY_NORMAL
        mov     cx,DISPLAY_WIDTH*DISPLAY_HEIGHT
        callsys 10h,9               ; BIOS write char - color screen

        mov     bh,DISPLAY_PAGE
        mov     dx,0
        callsys 10h,2               ; Set Cursor Position (BIOS)
        mov     ah,9
        mov     al,' '
        mov     bh,DISPLAY_PAGE
        mov     bl,DISPLAY_HILIGHT
        mov     cx,DISPLAY_WIDTH
        callsys 10h,9               ; BIOS write char - color top line

; set up title bar text
        mov     si,offset ptitle
        mov     dx,0                ; cursor position
        call    printString

; set up screen text borders
        mov     si,offset pbtop
        mov     dl,0                ; dl,dh (x,y)
        mov     dh,1
        call    printString

        mov     cx,MIDDLE_LINE-2
        mov     dh,2
__loop1:
        mov     si,offset pline
        mov     dl,0
        call    printString
        mov     si,offset pline
        mov     dl,DISPLAY_WIDTH-1
        call    printString
        inc     dh
        loop    __loop1

        mov     si,offset pbmid
        mov     dl,0
        call    printString

        mov     cx,(DISPLAY_HEIGHT-2)-MIDDLE_LINE
__loop2:
        mov     si,offset pline
        mov     dl,0
        inc     dh
        call    printString
        mov     si,offset pline
        mov     dl,DISPLAY_WIDTH-1
        call    printString
        loop    __loop2

        mov     si,offset pbbot
        mov     dl,0
        inc     dh
        call    printString
```

```
        mov     dl,43
        mov     si,offset pctop
        call    printString

        mov     cx,7
__loop3:
        mov     si,offset pline
        mov     dl,43
        inc     dh
        call    printString
        mov     si,offset pline
        mov     dl,DISPLAY_WIDTH-3
        call    printString
        loop    __loop3

        mov     dl,43
        inc     dh
        mov     si,offset pcbot
        call    printString

; make page the current display page
        ret



;
; --- Procedure: resetDisplay
; --- Input: none
; --- Output: none
; --- Preserves: none
;

resetDisplay:
        mov     al,0
        callsys 10h,5           ; BIOS Set Active Page back to zero
        ret



        end
; end of file 'main.asm'
```

```
;
; --- Program: o2volt
; --- File: util.asm
; --- Author: David Dahle (summer apprentice)
; --- Date: July-August 1992
; --- Purpose: misc routines
; --- Letterman Army Institute of Research
; --- Persidio of San Francisco, CA 94129-6800
;
;**********************************************************************

        dosseg
        locals
        include header.i


        .model  small


        .data
; string gadget variables
g_flags db      0               ; gadget flags 0=string 1=num
scrn_x  db      0
scrn_y  db      0               ; x,y position of gadget
scrn_w  db      0               ; onscreen width of gadget
buf_len db      0               ; characters in buffer
buf_off db      0               ; display offset into buffer
buf_cur db      0               ; cursor offset into buffer
buf_wid db      0               ; max length of buffer
buf_seg dw      0               ; segment buffer is in
buf_ptr dw      0               ; segment offset to buffer

; number to ascii text buffer
nconbuf db      NUMBUF_LEN DUP (0)

; dislay window parameters
curlin  db      0               ; cursor position in status window

; dos error texts
diskfer db      'Disk Full',0
derrmsg db      'Invalid function number                ',0
        db      'File not found                         ',0
        db      'Path not found                         ',0
        db      'No more handles (to many files)        ',0
        db      'Access denied                          ',0
        db      'Invalid handle                         ',0
        db      'Memory control blocks destroyed        ',0
        db      'Not enough memory                      ',0
        db      'Invalid memory-block address           ',0
        db      'Invalid environment block              ',0
        db      'Invalid format                         ',0
        db      'Invalid file-access code               ',0
        db      'Invalid data                           ',0
        db      'You should never see this one!!!?!@#!@ ',0
        db      'Invalid drive specification            ',0
        db      'Attempt to remove the current directory',0
        db      'Not the same device                    ',0
        db      'No more files                          ',0
MAX_DOSERROR_NUMBER     equ     18
DOSERROR_STRING_WIDTH   equ     40
CONFIG_LENGTH           equ     4+(FNAME_LEN*2)+NUMBUF_LEN+(2*5)

cnfoper db      'ERROR: Unable to open configuration file.',0
cnfrder db      'ERROR: DOS Error while reading configuration file.',0
cnfwter db      'ERROR: DOS Error while writing configuration file.',0
```

```
cnfwtok db      'Configuration File written.',0
cnfrdok db      'Configuration File Read.',0
cnfbuf  db      (CONFIG_LENGTH) DUP (0)
cnfanyl db      'Name of Configuration File: ',0
.

        global  nconbuf:byte,curdate:word,autonum:word,startup:byte
        global  blankl:byte


        .code

; labels in file 'main.asm'
        extrn   print_allConfig:near

;
; --- Procedure: getConfigFileName
; --- Input: none
; --- Output: ax=0, don't load file
; ---             ax!=0, load file, ds:dx=filename
; --- Preserves:
;

        public  getConfigFileName
getConfigFileName:
        mov     dl,3
        mov     dh,12
        lea     si,cnfanyl
        call    printString

        mov     al,0
        lea     bx,cnfbuf
        mov     [bx],al
        mov     cl,127
        mov     ch,40
        mov     dl,31
        mov     dh,12
        call    setUpStringGadget

mainLoop:
        callsys 16h,0           ; wait for next character

        cmp     al,0            ; check for extended characterr
        je      short __extended
        cmp     al,13           ; check for return key
        jne     short __notReturn
        call    removeStringGadget
        mov     cl,74
        mov     dl,3
        mov     dh,12
        lea     si,blankl
        call    printStringLimit
        mov     ax,1
        lea     dx,cnfbuf
        ret

__notReturn:
        cmp     al,8                    ; check for backspace
        jne     short __notBackspace
        call    backStringGadgetChar
        jmp     mainLoop
__notBackspace:
        cmp     al,27
        jne     short __notEscape
        call    removeStringGadget
```

```
        mov     dl,3
        mov     dh,12
        lea     si,blankl
        call    printStringLimit
        mov     ax,0
        ret
__notEscape:
        cmp     al,32                   ; a printable character?
        jl      short __notPrintChar
        cmp     al,126
        jg      short __notPrintChar
        call    addStringGadgetChar
        jmp     mainLoop
__notPrintChar:
        jmp     mainLoop

__extended:
        cmp     ah,75
        jne     short __notLeft         ; check for Cursor Left
        call    leftStringGadgetChar
        jmp     mainLoop
__notLeft:
        cmp     ah,77
        jne     short __notRight        ; check for Cursor Right
        call    rightStringGadgetChar
        jmp     mainLoop
__notRight:
        cmp     ah,83                   ; check for a delete
        jne     short __notDelete
        call    delStringGadgetChar
        jmp     mainLoop
__notDelete:
        jmp     mainLoop


;
; --- Procedure: loadConfigurationFile
; --- Input: dx=name of config file to load
; --- Output:
; --- Preserves:
;

        public  loadConfigurationFile
loadConfigurationFile:
        mov     al,0            ; open for reading
        callsys 21h,3dh
        jnc     short __noOpenError
        lea     si,cnfoper
        call    dosIOError
        jmp     __exit

__noOpenError:
        push    ax              ; save handle
        mov     bx,ax
        mov     cx,51           ; length of init string
        lea     dx,nconbuf      ; destination buffer
        callsys 21h,3fh
        jnc     __noFileError1
        lea     si,cnfrder
        call    dosIOError
        jmp     __closeExit

__noFileError1:
        cmp     ax,cx
```

```
        lea     si,cnfrder
        call    dosIOError
        jmp     __closeExit

__noFileError2:
        lea     di,startup
        mov     si,dx
__cmpLoop:
        mov     al,[si]         ; string read
        inc     si
        mov     ah,[di]         ; source string
        inc     di
        cmp     al,ah
        jne     short __notConfigFile
        cmp     ah,0            ; equal at null, string ok
        jne     short __cmpLoop

        pop     bx
        push    bx
        mov     cx,CONFIG_LENGTH
        lea     dx,cnfbuf
        callsys 21h,3fh
        jnc     short __noReadError1
        lea     si,cnfrder
        call    dosIOError
        jmp     short __closeExit

__noReadError1:
        cmp     ax,cx
        je      short __noReadError2
        lea     si,cnfrder
        call    dosIOError
        jmp     short __closeExit

__noReadError2:
        lea     si,cnfbuf
        lea     di,curdate
        mov     ax,[si]
        cmp     [di],ax
        je      short __dateSame1
        jmp     short __dateNotSame

__dateSame1:
        mov     ax,[si+2]
        cmp     [di+2],ax
        jne     short __dateNotSame
        mov     ax,[autonum]
        jmp     short __allHere

__dateNotSame:
        mov     ax,-1           ; set -1, reset autonum to 1

__allHere:
        add     si,4
        add     di,4
        mov     dx,ds
        mov     es,dx
        mov     cx,CONFIG_LENGTH-4
        cld
__copyLoop:
        movsb
        loop    __copyLoop
        cmp     ax,-1
        jne     short __noResetAuto
        mov     [autonum],1
```

```
__noResetAuto:
        call    print_allConfig
        lea     si,cnfrdok
        call    printTextToScreen
        jmp     __closeExit

__notConfigFile:
        lea     si,notcf
        call    printTextToScreen

__closeExit:
        pop     bx
        callsys 21h,3eh          ; close file handle

__exit:
        call    clearKeyBoardBuffer
        ret



;
; --- Procedure: saveConfigurationFile
; --- Input: dx=file name
; --- Output:
; --- Preserves:
;

        public  saveConfigurationFile
saveConfigurationFile:
        mov     cx,0
        callsys 21h,3ch
        jnc     short __noOpenError
        lea     si,cnfoper
        call    dosIOError
        jmp     short __exit

__noOpenError:
        push    ax
        mov     bx,ax

        lea     dx,startup
        mov     cx,51
        callsys 21h,40h
        jnc     short __noFileWriteError1
        lea     si,cnfwter
        call    dosIOError
        jmp     short __closeExit

__noFileWriteError1:
        cmp     ax,cx
        je      short __noFileWriteError2
        mov     al,-1
        call    dosIOError
        jmp     short __closeExit

__noFileWriteError2:
        pop     bx
        push    bx
        lea     dx,curdate
        mov     cx,CONFIG_LENGTH
        callsys 21h,40h
        jnc     short __noFileWriteError3
        lea     si,cnfwter
        call    dosIOError
        jmp     short __closeExit
```

```
__noFileWriteError3:
        cmp     ax,cx
        je      short __noFileWriteError4
        mov     al,-1
        call    dosIOError
        jmp     short __closeExit

__noFileWriteError4:
        lea     si,cnfwtok
        call    printTextToScreen

__closeExit:
        pop     bx
        callsys 21h,3eh

__exit:
        call    clearKeyBoardBuffer
        ret



;
; --- Procedure: dosIOError
; --- Input: si=pointer to initial string to output
; ---        al=error number returned by dos (-1=diskfull)
; --- Output:
; --- Preserves:
;

        public  dosIOError
dosIOError:
        push    ax
        call    printTextToScreen
        pop     ax
        cmp     al,-1
        jne     short __noDiskFullError
        lea     si,diskfer
        jmp     short __printText
__noDiskFullError:
        cmp     al,MAX_DOSERROR_NUMBER
        ja      short __noErrorMessage          ; unsigned, please
        dec     al
        mov     bl,DOSERROR_STRING_WIDTH
        mul     bl              ; error in al
        lea     si,derrmsg
        add     si,ax
__printText:
        call    printTextToScreen
        ret
__noErrorMessage:
        mov     ah,0
        call    convertItoA
        mov     si,bx
        call    printTextToScreen
        ret



;
; --- Procedure: clearKeyBoardBuffer
; --- Input: none
; --- Output: none
; --- Preserved: ...?
;
```

```
clearKeyBoardBuffer:
        callsys 16h,1
        je      short __noCharacter
        callsys 16h,0
        jmp     short clearKeyBoardBuffer
__noCharacter:
        ret



;
; --- Procedure: printTextToScreen
; --- Input: ds:si=points to a null-terminated string to output
; --- Output:
; --- Preserves:
;

        PUBLIC  printTextToScreen
printTextToScreen:
        mov     dl,1            ; x position to print
        mov     dh,[curlin]
        add     dh,MIDDLE_LINE+1
        mov     cl,78           ; width of screen we have
        call    printStringLimit

        inc     [curlin]
        cmp     [curlin],10
        jne     short __noNeedToScroll
        dec     [curlin]

        mov     al,1            ; number of lines to scroll
        mov     bh,DISPLAY_NORMAL       ; attribute of new blank line
        mov     cl,1
        mov     ch,MIDDLE_LINE+1
        mov     dl,78
        mov     dh,DISPLAY_HEIGHT-2
        callsys 10h,6

__noNeedToScroll:
        ret



;
; --- Procedure: printTtSBackLine
; --- Input:
; --- Output:
;

        public  printTtSBackLine
printTtSBackLine:
        cmp     [curlin],0
        je      short __atTopLine
        dec     [curlin]
__atTopLine:
        ret



;
; --- Procedure: setUpStringGadget
; --- Inputs: al=flags
;             ds:bx=pointer to buffer of an asciiz string
;             cl=max length of buffer including null
;             ch=length of onscreen display
```

```
; --- Output: none
; --- Preserves: ds,es,di
;

        public  setUpStringGadget
setUpStringGadget:
        mov     [g_flags],al        ; save parameters
        mov     [scrn_x],dl
        mov     [scrn_y],dh
        mov     [scrn_w],ch
        mov     [buf_wid],cl
        mov     [buf_seg],ds
        mov     [buf_ptr],bx

        mov     cl,0                ; get the number of chars in this buffer
__loop1:
        mov     al,[bx]
        inc     bx
        inc     cl
        cmp     al,0
        jne     __loop1
        dec     cl
        mov     [buf_len],cl
        mov     [buf_off],0                 ; set initial offsets
        mov     [buf_cur],0

        call    setDisplayAndCursorPos
        ret


;
; --- Procedure: setDisplayAndCursorPos
; --- Input: none
; --- Output: none
;

        public  setDisplayAndCursorPos
setDisplayAndCursorPos:
        push    ds
        mov     si,[buf_ptr]                ; buffer offset into segment
        mov     ds,[buf_seg]                ; buffer segment
        mov     al,[buf_off]
        mov     ah,0
        add     si,ax
        mov     cl,[scrn_w]                 ; gadget width
        mov     dl,[scrn_x]
        mov     dh,[scrn_y]
        call    printStringLimit
        pop     ds

        mov     dl,[scrn_x]
        add     dl,[buf_cur]
        mov     dh,[scrn_y]
        mov     bh,DISPLAY_PAGE
        callsys 10h,2                       ; BIOS set cursor pos
        ret


;
; --- Procedure: removeStringGadget
; --- Input: none
; --- Output: none
; --- Preserves: ??
```

```
        public  removeStringGadget
removeStringGadget:
        push    ds
        mov     si,[buf_ptr]
        mov     ds,[buf_seg]
        mov     cl,[scrn_w]
        mov     dl,[scrn_x]
        mov     dh,[scrn_y]
        call    printStringLimit
        pop     ds
        ret



;
; --- Procedure: addStringGadgetChar
; --- Input: al=ascii character to insert
; --- Output: none
;

        public  addStringGadgetChar
addStringGadgetChar:
        mov     cl,[buf_len]
        mov     ch,0
        inc     cx                      ; including null
        cmp     [buf_wid],cl            ; is the buffer already full
        jbe     __bufferFull            ; unsigned please!!!

        cmp     [g_flags],0             ; 0=string
        je      short __notNumberGadget

        cmp     al,'+'
        je      short __numberGadgetChar
        cmp     al,'-'
        je      short __numberGadgetChar
        cmp     al,'.'
        je      short __numberGadgetChar
        cmp     al,'0'
        jl      short __notNumberGadgetChar
        cmp     al,'9'
        jg      short __notNumberGadgetChar

__numberGadgetChar:
__notNumberGadget:
        push    ds
        push    es
        push    ax              ; save character
        mov     bl,[buf_off]
        add     bl,[buf_cur]
        mov     bh,0            ; bx=position in buffer

        mov     ax,[buf_seg]
        mov     ds,ax
        mov     es,ax
        mov     si,[buf_ptr]
        push    si
        add     si,cx           ; cx=characters in buffer
        dec     si              ; (old length)
        mov     di,si
        inc     di              ; destination address one byte later

        sub     cx,bx           ; cx=characters remaining

        std                     ; backwards!!
```

```
        loop    __loop

        pop     si
        pop     ax
        mov     [si+bx],al      ; bx=buffer pos, still
        pop     es
        pop     ds
        inc     [buf_len]
        call    setDisplayAndCursorPos
        call    rightStringGadgetChar

__bufferFull:
__notNumberGadgetChar:
        ret


;
; --- Procedure: delStringGadgetChar
; --- Input: none
; --- Output: none
;

        public  delStringGadgetChar
delStringGadgetChar:
        mov     al,[buf_len]
        mov     ah,[buf_off]
        add     ah,[buf_cur]
        cmp     al,ah
        jbe     __nothingToDelete

        push    ds
        mov     bx,[buf_seg]
        mov     ds,bx
        mov     es,bx
        mov     di,[buf_ptr]
        mov     cl,ah
        mov     ch,0
        add     di,cx

        mov     si,di
        inc     si
        sub     al,ah
        mov     cl,al
        mov     ch,0
        cld                     ; forwards
__loop: movsb
        loop    __loop

        dec     [buf_len]
        pop     ds
        call    setDisplayAndCursorPos

__nothingToDelete:
        ret


;
; --- Procedure: backStringGadgetChar
; --- Input: none
; --- Output: none
;

        public  backStringGadgetChar
```

```
        mov     al,[buf_cur]
        cmp     al,0
        jne     short __goFunction
        mov     al,[buf_off]
        cmp     al,0
        je      short __exit
__goFunction:
        call    leftStringGadgetChar
        call    delStringGadgetChar
__exit: ret


;
; --- Procedure: leftStringGadgetChar
; --- Input: none
; --- Output: none
;

        public  leftStringGadgetChar
leftStringGadgetChar:
        mov     al,[buf_cur]
        mov     ah,[buf_off]
        cmp     al,0
        jne     short __notCursorAtStart
        cmp     ah,0
        je      short __exit
        dec     ah
        jmp     short __setNewPos
__notCursorAtStart:
        dec     al
__setNewPos:
        mov     [buf_cur],al
        mov     [buf_off],ah
        call    setDisplayAndCursorPos
__exit: ret


;
; --- Procedure; rightStringGadgetChar
; --- Input: none
; --- Output: none
;

        public  rightStringGadgetChar
rightStringGadgetChar:
        mov     bl,[buf_len]
        mov     bh,[buf_off]
        mov     al,[buf_cur]
        mov     ah,[scrn_w]
        mov     cl,bh           ; is the cursor at the end of the text
        add     cl,al
        cmp     cl,bl
        je      short __exit    ; equal, at the end of the line
        inc     al              ; advance cursor pos by movement or scrolling
        cmp     al,ah
        jb      short __notAtTheEnd
        mov     al,ah
        dec     al              ; max pos is under the last character
        inc     bh              ; buffer offset
__notAtTheEnd:
        mov     [buf_off],bh    ; save new parameters
        mov     [buf_cur],al
        call    setDisplayAndCursorPos
```

```
;
; --- Procedure: printString / printCursorString
; --- Input: si=segment offset to null terminated string
; ---        (dl,dh=(x,y) position to start at)
; --- Ouput: si=points to next byte after string's null
; --- Preserves: bx,cx,dh,di,bp,ds,es
;

        public  printCursorString,printString
printCursorString:
        push    cx
        push    bx
        mov     bh,DISPLAY_PAGE
        callsys 10h,3               ; get current cursor pos
        pop     bx
        pop     cx                  ; ch,cl gets set to new cursor mode

printString:
        push    cx
        push    bx
        mov     bh,DISPLAY_PAGE
        callsys 10h,2               ; already in dx - cursor pos

__loop:
        mov     al,[si]             ; character to print
        inc     si
        cmp     al,0                ; zero, end of string
        je      short __end
        mov     bh,DISPLAY_PAGE
        mov     cx,1
        callsys 10h,0ah             ; BIOS print single character

        inc     dl
        mov     bh,DISPLAY_PAGE
        callsys 10h,2               ; BIOS set cursor pos
        jmp     __loop

__end:  pop     bx
        pop     cx
        ret



;
; --- Procedure: printStringAttrib /printCursorStringAttrib
; --- Input: si=segment offset to null terminated string
; ---        bl=attribute to make string
; ---        (dl,dh=(x,y) position to start at)
; --- Ouput: si=points to next byte after string's null
; --- Preserves: cx,dh,di,ds,es
;

        public  printCursorStringAttrib,printStringAttrib
printCursorStringAttrib:
        push    cx
        mov     bh,DISPLAY_PAGE
        callsys 10h,3               ; get current cursor pos
        pop     cx

printStringAttrib:
        push    cx                  ; save cx register
        mov     bh,DISPLAY_PAGE
```

```
__loop:
        mov     al,[si]             ; character to print
        inc     si
        cmp     al,0                ; zero, end of string
        je      short __end
        mov     bh,DISPLAY_PAGE
        mov     cx,1
        callsys 10h,09h             ; BIOS print single character

        inc     dl
        mov     bh,DISPLAY_PAGE
        callsys 10h,2               ; BIOS set cursor pos
        jmp     __loop

__end:  pop     cx
        ret



;
; --- Procedure: printStringLimit / printCursorStringLimit
; --- Input: si=segment offset to null terminated string
; ---        cl=length of string to print (pad with ' ' if shorter)
; ---        (dl,dh=(x,y) position to start at)
; --- Ouput: none
; --- Preserves: dh,di,ds,es
;

        public  printCursorStringLimit,printStringLimit
printCursorStringLimit:
        push    cx
        mov     bh,DISPLAY_PAGE
        callsys 10h,3               ; get cursor pos
        pop     cx

printStringLimit:
        mov     ch,0
        push    cx                  ; save cx register
        mov     bp,sp               ; to base ptr register
        mov     bh,DISPLAY_PAGE
        callsys 10h,2               ; already in dx - cursor pos

__loop:
        mov     al,[si]             ; character to print
        cmp     al,0                ; zero, end of string
        jne     short __jmp1
        mov     al,' '
        jmp     short __jmp2
__jmp1: inc     si
__jmp2: mov     bh,DISPLAY_PAGE
        mov     cx,1
        callsys 10h,0ah             ; BIOS print single character

        inc     dl
        mov     bh,DISPLAY_PAGE
        callsys 10h,2               ; BIOS set cursor pos

        dec     word ptr [bp]
        cmp     word ptr [bp],0
        jne     __loop               ; test if we shoud continue

        pop     cx
        ret
```

```
;
; --- Procedure: convertItoA / convertLtoA
; --- Input: (dx):ax=unsigned (long)word to convert
; --- Output: ds:bx=pointer to an asciiz string
; --- Preserves: si,bp,ds,es
;

        public  convertItoA,convertLtoA
convertItoA:
        mov     dx,0
convertLtoA:
        push    ax
        push    dx

; get the number of characters we will need to convert this number
        mov     bx,10
        mov     cx,0
__loop1:
        div     bx
        mov     dx,0
        inc     cx
        cmp     ax,0
        jne     __loop1

; convert this string to a ASCII string (base(bx)=10)
        pop     dx
        pop     ax
        mov     di,offset nconbuf
        add     di,cx
        mov     byte ptr [di],0       ; null terminate string
__loop2:
        div     bx
        add     dx,'0'
        dec     di                ; pre-deincrement mode -(di)
        mov     [di],dl           ; remainder will be less than 10
        mov     dx,0
        cmp     ax,0
        jne     __loop2
        mov     bx,offset nconbuf        ; return value
        ret


;
; --- Procedure: convertAtoI
; --- Input: ds:si=asciiz string to convert to an unsigned int
; --- Output: ax=unsigned word
; --- Preserves: di,si,ds,es
;
; Note: ignores '-','+', and '.' characters
;

        public  convertAtoI
convertAtoI:
; find the end of this string
        mov     cx,0
__loop1:
        mov     dl,[si]
        inc     cx
        inc     si
        cmp     dl,0
        jne     __loop1

; convert this string into a number
        mov     bx,1              ; base offset value
```

```
        dec     si
        sub     sp,2
        mov     bp,sp
        mov     word ptr [bp],0
        jcxz    __exit
__loop2:
        dec     si
        mov     ax,0
        mov     al,[si]
        cmp     al,'0'
        jl      short __notNumberChar
        cmp     al,'9'
        jg      short __notNumberChar
        sub     al,'0'
        mul     bx
        add     [bp],ax
        mov     ax,10
        mul     bx
        mov     bx,ax
__notNumberChar:
        loop    __loop2

__exit:
        pop     ax
        ret


        end
; end of file 'util.asm'
```

```
;
; --- Program: o2volt
; --- File: hard.asm
; --- Author: David Dahle (summer apprentice)
; --- Date: July-August 1992
; --- Purpose: routines interfacing us with the A/D converter
; --- Letterman Army Institute of Research
; --- Persidio of San Francisco, CA 94129-6800
;
;*********************************************************************

        dosseg
        locals
        include header.i

        .model  small

        .data
; error text
A       dw      16 dup(0)       ; 2-byte integer array of 16 elements
B       db      64 dup(0)       ; 4-byte readl array of 16 elements
C       db      10 dup(0)       ; 10 bytes of string space
; Change these numbers if more channels are added ------------
SN      db      8               ; number of analog channels
SM      db      16              ; number of digital I/O's
; -----------------------------------------------------------
avrhand dw      0               ; average file handle
dathand dw      0               ; data file handle
memhand dw      0               ; memory where data in read into
cursam  dw      0               ; number of the current sample
numread dw      0               ; number of readings taken
strtime dw      0,0             ; starting time
endtime dw      0,0             ; ending time
; messages
nocrd   db      'FATAL ERROR: Driver, ADRIVE.COM, not installed,'
        db      ' or analog card not installed.',0
notsel  db      'FATAL ERROR: No analog card selected.  BRD SEL'
        db      ' switch set to 0.',0
nocal   db      'FATAL ERROR: CALIB.DAT file not correct or FIND.EXE'
        db      ' was not run.',0
nocnos  db      'FATAL ERROR: Calibration numbers are not correct.',0
mchan   db      'FATAL ERROR: Channel count incorrect. Change variables'
        db      ' SN and SM in hard.asm.',0
malloce db      'ERROR: Unable to allocate read buffer.',0
avrfer  db      'ERROR: Unable to open specified average file'
        db      ' for writing.',0
datfer  db      'ERROR: Unable to open specified data file for'
        db      ' writing.',0
awrter  db      'ERROR: Uanble to write to destination average file.',0
dwrter  db      'ERROR: Unable to write to destination data file.',0
bufover db      'WARNING: Data Recieve Buffer overflow, data may be lost',0
ttllow  db      'WARNING: TTL signal already active, waiting for inactive.',0
colterm db      'Data Acquisition Completed.',0
strbuf  db      128 DUP (0)

        global  resol:byte,nconbuf:byte,avrfl:byte,datfl:byte,range:word
        global  etime:word,autonum:word,autofn:word,curdate:word

        .code
; labels in file 'main.asm'
        extrn   print_datfl:near,print_cmdLine:near,print_cmdLineBusy:near
```

```
; labels in file 'util.asm'
        extrn   printTtSBackLine:near,printTextToScreen:near
        extrn   dosIOError:near,clearKeyBoardBuffer:near

; labels in file 'fmath.c'
        extrn   _createInstantDisplayLine:near,_mkdfname:near
        extrn   _createStatusString:near,_computeAverage:near
        extrn   _convertFtoA:near,_createTimeTakenString:near
        extrn   _createAutomaticFileName:near,_insertAutoFileName:near

;
; --- Procedure: setUpHardware
; --- Input: none
; --- Output: ax=0 if no error
;             ax=1 if error, ds:dx points to error text
; --- Preserves:
;

        public  setUpHardware
setUpHardware:
        mov     [C],'F'         ;driver commands: read CALIB file
        mov     [C+1],'n'       ;get no. of channels
        mov     [C+2],0         ;end with null
        call    driver_CMD      ;call driver
        mov     ax,word ptr [A]         ;1st integer element returned
        mov     bx,word ptr [A+4]       ;3rd integer element returned
        mov     cx,word ptr [A+12]      ;7th integer element returned
        cmp     ax,0
        jne     short __set2
        cmp     bx,0
        jne     short __set1
        mov     dx,offset nocrd         ;error
        jmp     short __serr
__set1: mov     dx,offset notsel        ;error
        jmp     short __serr
__set2: cmp     cx,0
        jne     short __set3
        mov     dx,offset nocal         ;error
        jmp     short __serr
__set3: cmp     ax,cx
        jle     short __set4
        mov     dx,offset nocnos        ;error
        jmp     short __serr
__set4: cmp     ax,16
        jg      short __set5            ;error
        cmp     bx,offset SM
        jle     short __set6
__set5: mov     bx,offset mchan         ;error
        jmp     short __serr
__set6: mov     ax,0
        ret                             ;no error in setup
__serr: mov     ax,-1
        ret                             ;error in setup


;
; --- Procedure: beginDataAcquisition
; --- Input:
; --- Output:
; --- Preserves: nothing
;

        public  beginDataAcquisition
```

```
        call    initializeHardware
        call    print_cmdLineBusy

        cmp     [autofn],2
        je      short __noAutoFileName
        mov     ax,[autonum]
        push    ax
        lea     ax,curdate
        push    ax
        lea     ax,strbuf
        push    ax
        call    _createAutomaticFileName
        add     sp,6

        lea     ax,strbuf
        push    ax
        lea     ax,avrfl
        push    ax
        call    _insertAutoFileName
        add     sp,4
        call    print_avrfl
        lea     ax,strbuf
        push    ax
        lea     ax,datfl
        push    ax
        call    _insertAutoFileName
        add     sp,4
        call    print_datfl

__noAutoFileName:
        mov     bx,(DACQ_ENTRIES*4)/16  ; room for DACQ_ENTRIES num of readings
        callsys 21h,48h                 ; allocate memory
        jnc     __memoryAllocated       ; returns SEGMENT pointer
        lea     si,malloce
        call    dosIOError
        jmp     __exitDataAcq

__memoryAllocated:
        mov     [memhand],ax    ; save memory segment pointer

        cmp     [avrfl],0
        je      short __noAvrFileNec

        lea     dx,avrfl
        mov     cx,0
        callsys 21h,3ch         ; Create new/Replace old file
        jnc     __avrFileOpened
        lea     si,avrfer
        call    dosIOError
        jmp     __exitNoAvrFile

__avrFileOpened:
        mov     [avrhand],ax

__noAvrFileNec:
        mov     [cursam],1              ; init current sample number

__dataAcq_MainLoop:
        cmp     [datfl],0
        je      short __noDatFileNec

        mov     ax,[cursam]
        push    ax
        lea     ax,[datfl]
        push    ax
```

```
        add     sp,4
        call    print_datfl

        lea     dx,datfl
        mov     cx,0                    ;create new file
        callsys 21h,3ch         ; Create new/Replace old file
        jnc     short __datFileOpened
        lea     si,datfer
        call    dosIOError
        jmp     __exitErrorDatFile

__datFileOpened:
        mov     [dathand],ax

__noDatFileNec:
        mov     [C],'c'
        mov     [C+1],0
        call    driver_CMD      ; re-calibrate card
        mov     dx,0            ; flag to make sure we start inactive
__waitingForTTL:
        callsys 16h,1           ; character ready
        je      short __waitingNoCharacter
        lea     si,colterm
        call    printTextToScreen
        jmp     __endDataAcquisition
__waitingNoCharacter:
        mov     [C],'I'
        mov     [C+1],0
        call    driver_CMD
        cmp     [A],0
        je      short __signalActiveNow
        mov     dx,1            ; found atleast once in inactive state
        jmp     __waitingForTTL

__signalActiveNow:
        cmp     dx,1
        je      short __foundInactive
        lea     si,ttllow
        call    printTextToScreen       ; output error
__waitForInactiveLoop:
        callsys 16h,1           ; character ready
        je      short __waitNoChar
        lea     si,colterm
        call    printTextToScreen
        jmp     __endDataAcquisition
__waitNoChar:
        mov     [C],'I'
        mov     [C+1],0
        call    driver_CMD
        cmp     [A],0
        je      short __waitForInactiveLoop
        jmp     short __noDatFileNec

__foundInactive:
        callsys 21h,2ch                 ; get current time
        mov     byte ptr [strtime],ch
        mov     byte ptr [strtime+1],cl
        mov     byte ptr [strtime+2],dh
        mov     byte ptr [strtime+3],dl

        mov     ax,[memhand]            ; set registers for movsw
        mov     es,ax
        mov     di,0
        mov     [numread],0
__readValuesLoop:
```

```
        jb      short __receiveBufNotFull
        lea     si,bufover
        call    printTextToScreen
        jmp     short __dataSetCompleted
__receiveBufNotFull:
        inc     [numread]
        mov     [A],1
        mov     [C],'h'
        mov     [C+1],0
        call    driver_CMD
        cld
        lea     si,B                    ; where value is
        movsw
        movsw                           ; move float
        mov     [C],'I'
        mov     [C+1],0
        call    driver_CMD
        cmp     [A],0
        je      __readValuesLoop        ; keep reading values

__dataSetCompleted:
        callsys 21h,2ch                 ; get time
        mov     byte ptr [endtime],ch
        mov     byte ptr [endtime+1],cl
        mov     byte ptr [endtime+2],dh
        mov     byte ptr [endtime+3],dl

        mov     ax,[numread]
        push    ax                      ; number read
        push    es
        mov     ax,0                    ; far pointer to buffer
        push    ax
        lea     ax,nconbuf
        push    ax                      ; buffer
        call    _computeAverage
        add     sp,8

        mov     ax,[numread]
        push    ax
        mov     ax,[cursam]
        push    ax
        lea     ax,nconbuf
        push    ax
        lea     ax,strbuf
        push    ax
        call    _createStatusString
        add     sp,8
        lea     si,strbuf
        call    printTextToScreen

        cmp     [etime],2
        je      short __noPrintTimeTaken
        mov     ax,[numread]
        push    ax
        lea     ax,endtime
        push    ax
        lea     ax,strtime
        push    ax
        lea     ax,strbuf
        push    ax
        call    _createTimeTakenString
        add     sp,8
        lea     si,strbuf
        call    printTextToScreen
```

```
        cmp     [avrfl],0
        je      short __noAvrFileWrite
        lea     dx,nconbuf
        call    setStringForWrite
        mov     bx,[avrhand]
        callsys 21h,40h
        jnc     short __noAvrWriteError
        lea     si,awrter
        call    dosIOError
        jmp     __endDataAcquisition
__noAvrWriteError:
        cmp     ax,cx
        je      short __noAvrWriteError2
        mov     al,-1
        lea     si,awrter
        call    dosIOError
        jmp     __endDataAcquisition
__noAvrWriteError2:

__noAvrFileWrite:
        cmp     [datfl],0
        je      short __noDatFileWrite
        mov     cx,0
        mov     bx,0
__datFileWriteLoop:
        push    bx
        push    cx
        mov     ax,[memhand]
        mov     es,ax
        lea     ax,nconbuf
        push    ax
        lea     di,B
        mov     ax,es:[bx]
        mov     [di],ax
        mov     ax,es:[bx+2]
        mov     [di+2],ax
        push    di
        call    _convertFtoA
        add     sp,4

        lea     dx,nconbuf
        call    setStringForWrite
        mov     bx,[dathand]
        callsys 21h,40h
        mov     dx,cx
        pop     cx
        pop     bx
        jnc     short __noDatWriteError
        lea     si,dwrter
        call    dosIOError
        jmp     __endDataAcquisition
__noDatWriteError:
        cmp     dx,ax
        je      short __noDatWriteError2
        mov     al,-1
        lea     si,dwrter
        call    dosIOError
        jmp     __endDataAcquisition
__noDatWriteError2:
        add     bx,4
        inc     cx
        cmp     [numread],cx
        jne     short __datFileWriteLoop
```



```
__noDatFileWrite:
```

```
        mov     bx,[dathand]
        callsys 21h,3eh                 ; close data file
        jmp     __dataAcq_MainLoop

__endDataAcquisition:
        cmp     [autofn],2
        je      short __noAutoExpInc
        mov     ax,[autonum]
        cmp     ax,99
        je      short __numberNoCarry
        inc     ax
        jmp     short __numberCarryContinue
__numberNoCarry:
        mov     ax,1
__numberCarryContinue:
        mov     [autonum],ax
        call    print_autonum
__noAutoExpInc:
        cmp     [datfl],0
        je      short __exitErrorDatFile
        mov     bx,[dathand]
        callsys 21h,3eh         ; close file handle
        lea     dx,datfl
        callsys 21h,41h         ; delete file

__exitErrorDatFile:
        cmp     [avrfl],0
        je      short __exitNoAvrFile
        mov     bx,[avrhand]
        callsys 21h,3eh

__exitNoAvrFile:
        mov     ax,[memhand]
        mov     es,ax
        callsys 21h,49h

__exitDataAcq:
        call    clearKeyBoardBuffer
        call    print_cmdLine
        ret


;
; --- Procedure: setStringForWrite
; --- Input:
; --- Output:
; --- Preserves:
;

setStringForWrite:
        mov     bx,dx
        mov     cx,0
__strLengthLoop:
        mov     al,[bx]
        cmp     al,0
        je      short __endOfString
        inc     cx
        inc     bx
        jmp     short __strLengthLoop
__endOfString:
        mov     byte ptr [bx],13
        inc     cx
        mov     byte ptr [bx+1],10
        inc     cx
```

```
;
; --- Procedure: instant_Readings
; --- Input: none
; --- Output: none
; --- Preserves:
;

        public  instant_Readings
instant_Readings:
        call    initializeHardware
        call    print_cmdLineBusy

        mov     [C],'c'
        mov     [C+1],0
        call    driver_CMD

__loop:
        mov     [C],'I'
        mov     [C+1],0
        call    driver_CMD
        mov     ax,[A]
        push    ax

        mov     [A],1
        mov     [C],'h'
        mov     [C+1],0
        call    driver_CMD

        lea     ax,nconbuf
        push    ax
        lea     ax,B
        push    ax
        call    _createInstantDisplayLine
        add     sp,6
        lea     si,nconbuf
        call    printTextToScreen

        callsys 16h,1           ; check if character is ready
        jne     short __exitLoop
        call    printTtSBackLine
        jmp     short __loop

__exitLoop:
        call    clearKeyBoardBuffer
        call    print_cmdLine
        ret


;
; --- Procedure: initializeHardware
; --- Input: none
; --- Output: none
; --- Preserves:
;

        PUBLIC  initializeHardware
initializeHardware:
        mov     ax,1            ; use one analog channel
        mov     word ptr [A],ax
        mov     [C],'N'         ; set number of analog input channels
        mov     [C+1],0
```

```
        mov     al,[resol]
        cmp     al,1
        jne     short __notResol1
        mov     ax,18               ; low noise mode
        jmp     short __endResol
__notResol1:
        cmp     al,2
        jne     short __notResol2
        mov     ax,16               ; 16-bit mode
        jmp     short __endResol
__notResol2:
        cmp     al,3
        jne     short __notResol3
        mov     ax,15               ; 15-bit mode
        jmp     short __endResol
__notResol3:
        cmp     al,4
        jne     short __notResol4
        mov     ax,14               ; 14-bit mode
        jmp     short __endResol
__notResol4:
        cmp     al,5
        jne     short __notResol5
        mov     ax,13               ; 13-bit mode
        jmp     short __endResol
__notResol5:
        mov     ax,12               ; 12-bit mode
__endResol:
        mov     [A],ax
        mov     [C],'a'             ; Set resolution of all analog input channels
        mov     [C+1],0
        call    driver_CMD

        mov     ax,[range]
        mov     [A],ax
        mov     [C],'r'             ; Set range of analog input
        mov     [C+1],'c'
        mov     [C+2],0
        call    driver_CMD

        mov     ax,0                ; set for input (0) (1=output)
        mov     [A],ax
        mov     [C],'S'             ; set Digital I/O to input or output
        mov     [C+1],0
        call    driver_CMD

        mov     ax,0                ; set channel switch delay time to 0
        mov     [A],ax
        mov     [C],'d'
        mov     [C+1],0
        call    driver_CMD
        ret


;
; --- Procedure: driver_CMD
; --- Input: Arrays A,B, and C initialized with command and parameters
; --- Output:
; --- Preserves: all general purpose registers
;
```



```
driver_CMD:
        push    bp
```

```
        mov     ax,offset A
        push    ax
        push    ds
        mov     ax,offset B
        push    ax
        push    ds
        mov     ax,offset C
        push    ax
        mov     bp,sp
        call    check                   ;check if driver is there
        cmp     ax,-1
        jz      short __ndrv
        cld                             ; clear direction flag
        cli
        mov     ax,'CA'                 ;put mark in a reg
        int     60H                     ;call trap 60h to do the command
        nop                             ;just for leg room
        sti                             ;turn interrrups on
__ndrv: add     sp,12                   ;correct stack pointer
        pop     bp
        ret

; check to make sure the driver is actutally present
check:  push    si
        push    es
        mov     ax,0
        mov     es,ax
        mov     ax,es:[060h*4]
        cmp     ax,0
        je      short __ndrv
        mov     si,ax
        mov     ax,es:[060h*4+2]
        cmp     ax,0
        je      short __ndrv
        mov     es,ax
        mov     al,es:[si]
        cmp     al,03dh
        jne     short __ndrv
        mov     ax,0
        pop     es
        pop     si
        ret
__ndrv: mov     ax,-1
        pop     es
        pop     si
        ret


        end
; end of file 'hard.asm'
```

```
*
* --- Program: o2volt
* --- File: fmath.c
* --- Author: David Dahle (summer apprentice)
* --- Date: July-August 1992
* --- Purpose: floating point math and string routines
* --- Letterman Army Institute of Research
* --- Persidio of San Francisco, CA 94129-6800
*
**********************************************************************/


#include <stdlib.h>
#include <stdio.h>
#include <string.h>
#include <dos.h>


struct dosdate_me {
        unsigned int year;
        unsigned char day;
        unsigned char month;
};


/*
 * insertAutoFileName - inserts the date string into the given file name
 *
 * file=name string to insert into
 * date=date string to insert
 */

int insertAutoFileName(char *file,char *date)
{
        char ext[127], c;
        signed int i;

        ext[0]=0;
        if (file[0]==0) goto enda;

        for (i=strlen(file); (i);) {
                c=file[--i];
                if (c=='.') {
                        strcpy(ext,&file[i]);
                        file[i]=0;
                } else if (c=='\\') {
                        strcpy(&file[i+1],date);
                        goto endf;
                }
        }
        strcpy(file,date);
endf :  strcat(file,ext);
enda :  return 0;
}


/*
 * createAutomaticFileName - creates a string for the automatic file name
 *                              feature
 *
 * buf=destination buffer for string
 * date=pointer to date structure
 * experiemnt=number of the current experiment
 */
```

```
{
        sprintf(buf,"%02d%02d%02d%02d",
        (int)date->month,(int)date->day,(int)(date->year-1900),experiment);
}


/*
 * createTimeTakenString - compute time and hertz and create display string
 *
 * strbuf=destination buffer for output string
 * strtime=first time reading
 * endtime=second time reading
 * numread=number of readings taken
 */

void createTimeTakenString(char *strbuf,struct dostime_t *strtime,
                                          struct dostime_t *endtime,int numread)
{
        signed char s,h;
        unsigned char buf[20];
        double time;
        int hertz;

        s=(signed char)endtime->second-strtime->second;
        h=(signed char)endtime->hsecond-strtime->hsecond;
        if (h<0) {
                h=h+100;
                s--;
        }
        sprintf(buf," %d.%02dE+000",(int)s,(int)h);
        time=atof(buf);
        if (!time)
                hertz=0;
        else
                hertz=(int) ((double)numread/time);

        sprintf(strbuf,"Time: %d.%02d seconds. Hertz: %d",
                                                  (int)s,(int)h,hertz);
}


/*
 * convertFtoA - converts a float to an asciiz string for printing
 *
 * variable=pointer to a float
 * buffer=destination buffer for asciiz string
 */

void convertFtoA(float *variable,char *buffer)
{
        sprintf(buffer," %e",*variable);
}


/*
 * createStatusString - create status string for printing
 *
 * buffer=destination for string
 * average=pointer to a string for the average
 * cursam=current set number
 * numread=readingds in the current set
 */

void createStatusString(char *buffer,char *average,int cursam, int numread)
{
```

```
                                                    numread,cursam,average);
}


/*
 * computeAverage - computes the average of all the o2 voltages read
 *
 * buffer=destination buffer for string
 * values=far pointer (segment:offset passed) to the buffer of floats
 * numread=number of entries in the values buffer
 */

void computeAverage(char *buffer,float far *values,unsigned int numread)
{
        register i;
        double average,temp;

        for (i=0; i<numread; i++) {
                temp=(double)values[i]; /* does it do this anyway??? */
                average=average+temp;
        }
        temp=(double)numread;
        average=average/temp;
        sprintf(buffer," %e",average);
}


/*
 * createInstantDisplayLine - create string for instant function
 *
 * variable=pointer to the float that was just read
 * buffer=destination for string
 * digit=state of digital input
 */

void createInstantDisplayLine(float *variable,char *buffer,int digit)
{
        char    digs[5];

        if (digit==1)
                strcpy(digs,"HIGH");
        else
                strcpy(digs,"LOW");
        sprintf(buffer,"Voltage: %e    Digital: %s",*variable,digs);
}


/*
 * mkdfname - adds an extension to the oxygen voltage reading file
 *              based on the number of the SET that we are on
 *
 * name=name of the file (without extension)
 * cursam=SET number we are on
 */

void mkdfname(char *name, int cursam)
{
        register int i;
        char ext[4];

        sprintf(&ext[0],"%03d",cursam);

        for (i=0; (1); i++) {
                if (name[i]=='.') {
                        strcpy(&name[i+1],&ext[0]);
```

```
            } else if (name[i]==0) {
                    name[i]='.';
                    strcpy(&name[i+1],&ext[0]);
                    break;
            }
        }
}


/* end of file 'fmath.c' */
```

Appendix C

Program that processes spectral data from the spectrophotometric system.

```
#include <stdio.h>
#include <stdlib.h>
#include <math.h>
#include <signal.h>
#include <ctype.h>
#include <dos.h>
#include <string.h>
#include <io.h>
#include <fcntl.h>
#include <sys\types.h>
#include <sys\stat.h>

#define MAX_LINE_BUF    256
#define FILEBUF_SIZE    0x800
#define FALSE           0
#define TRUE            1

int     exitflag=FALSE;               /* flag set when a CTRL-C is hit */
char    sourcefn[50],destfn[50];      /* source/dest file names */
char    linebuffer[FILEBUF_SIZE];     /* output buffer for writing to file */
int     linebuf_size;                 /* number of chars currently in buffer */

/*
 * Usage - printf switch options to the screen
 */

void Usage(char **argv)
{
        printf("\nUsage: %s [switches]\n",argv[0]);
        printf("    -b[number]   starting wavelength\n");
        printf("    -d[filename] destination file name\n");
        printf("    -e[number]   ending wavelength\n");
        printf("    -n[number]   samples per file\n");
        printf("    -s[filename] source file name\n");
        printf("    -w[1|2]      read whole number wavelengths or all\n");
        printf("                1=read only whole number wavelngths\n");
        printf("                2=read all wavelgnths\n");
}


/*
 * chandler - Control-C interrupt handler. sets the exitflag=TRUE
 */

int chandler(void)
{
        signal(SIGINT, SIG_IGN);          /* disallow Ctr-C during handler */
        exitflag=TRUE;
        signal(SIGINT,chandler);          /* reattach handler to CTR-C */
        return 0;
}


/*
 * readline - read in a block of text ending with a linefeed '\n'=10
 */

int readline(int source,char *linebuf)
{
        register int x=0;
        do {    if (read(source,&linebuf[x],1)!=1) {
                        sprintf(linebuf,"Error reading '%s'",sourcefn);
                        perror(linebuf);
                        return 1;
```

```
	} while (linebuf[x++]!='\n');
	linebuf[x]=0;
	return 0;
}

/*
 * writeline - write file data to disk
 */

int writeline(int filehandle)
{
	if ((write(filehandle,linebuffer,linebuf_size))==-1) {
		sprintf(linebuffer,"\nError writing to '%s'",destfn);
		perror(linebuffer);
		return 1;
	}
	linebuf_size=0;
	return 0;
}

/*
 * saveline - writes the current data to memory for a future disk write
 */

int saveline(int filehandle,char *linebuf,int length)
{
	register int x=0;
	if (length+linebuf_size > FILEBUF_SIZE) {
		if (writeline(filehandle)) return 1;
		linebuf_size=0;
	}
	while (x<length)
		linebuffer[linebuf_size++]=linebuf[x++];
	return 0;
}

/*
 * main -
 */

int main(int argc,char **argv)
{
	char linebuf[MAX_LINE_BUF];	/* file read buffer */
	char absorbance[25];		/* buffer for reading in absorbance */
	int source=NULL,dest=NULL;	/* file handles */
	int startwvlen=0,endwvlen=0;	/* starting end ending wavelengths */
	int curwvlen,cwvlen_dec;	/* current wavelength we are at */
	int fstwvlen,fendwvlen;		/* wavelengths in current file */
	int fstwvl_dec,fendwvl_dec;	/* decimal of wavelengths in file */
	int cwvlen_val;			/* value of offset for fseek calc. */
	int incwvlen=0;			/* do we read only whole no. wl */
	int samplecount=0;		/* number of sets in this file */
	struct dostime_t time1, time2; /* variables used for computing time */
	int m,s;			/* more variables for computing time */
	register int x;			/* general purpose indez variable */

	sourcefn[0]=destfn[0]=0;	 /* null file names */

	/* set CTR-C handler so we can exit properly */
	if (signal(SIGINT,chandler)==(int(*) ())-1) {
		printf("ERROR: Unable to set CTR-C handler");
		goto end;
	}

	/* process switches */
```

```
	if (!(argv[x][0]=='-')) {
		printf("ERROR: Switch error\n");
		Usage(argv);
		goto end;
	} else {
		switch(tolower(argv[x][1])) {
			case 'b' :
				startwvlen=(int)atoi(&argv[x][2]);
				break;
			case 'd' :
				strcpy(destfn,&argv[x][2]);
				break;
			case 'e' :
				endwvlen=(int)atoi(&argv[x][2]);
				break;
			case 'n' :
				samplecount=(int)atoi(&argv[x][2]);
				break;
			case 's' :
				strcpy(sourcefn,&argv[x][2]);
				break;
			case 'w' :
				incwvlen=(int)atoi(&argv[x][2]);
				if (!(incwvlen==1 || incwvlen==2)) {
					printf("ERROR: -w switch: Invalid number given\n");
					goto end;
				}
				break;
			case '?' :
				 Usage(argv);
				 goto end;
			default :
				printf("ERROR: Unknown Switch '%c'\n",argv[x][1]);
				Usage(argv);
				goto end;
		}
	}
}

/* if the user hasn't already given the parameters, get them now */
if (exitflag) { printf("\n ** Aborted **\n"); goto end; }
if (!sourcefn[0]) {
		printf("Name of file with .ASC data file: ");
		scanf("%s",sourcefn);
} else printf("Name of file with .ASC data file: %s\n",sourcefn);
if (exitflag) { printf("\n ** Aborted **\n"); goto end; }
if (!destfn[0]) {
		printf("Name of file to create: ");
		scanf("%s",destfn);
} else printf("Name of file to create: %s\n",destfn);
if (exitflag) { printf("\n ** Aborted **\n"); goto end; }
if (!samplecount) {
		printf("Number of samples in this file: ");
		scanf("%d",&samplecount);
} else printf("Number of samples in this file: %d\n",samplecount);
if (exitflag) { printf("\n ** Aborted **\n"); goto end; }
if (!startwvlen) {
		printf("Starting wavelength: ");
		scanf("%d",&startwvlen);
} else printf("Starting wavelength: %d\n",startwvlen);
if (exitflag) { printf("\n ** Aborted **\n"); goto end; }
if (!endwvlen) {
		printf("Ending wavelength: ");
		scanf("%d",&endwvlen);
} else printf("Ending wavelength: %d\n",endwvlen);
```

```
if (!incwvlen) {
        printf("Read only whole number wavelengths (y|n):   ");
loop2:  if (exitflag) { printf("\n ** Aborted **\n"); goto end; }
        incwvlen=(int)getchar();
        if (incwvlen=='y')
                incwvlen=1;
        else if (incwvlen=='n')
                incwvlen=2;
        else    goto loop2;
} else if (incwvlen==1)
                printf("Reading only whole number wavelengths.\n");
  else printf("Reading all wavelengths.\n");
if (exitflag) { printf("\n ** Aborted **\n"); goto end; }

if (startwvlen>endwvlen) {
        printf("\nERROR: Starting wavelength > Ending wavelength\n");
        goto end;
}
source=open(sourcefn,O_BINARY|O_RDONLY,0);
if (source==-1) {
        sprintf(linebuf,"Unable to open '%s': ",sourcefn);
        perror(linebuf);
        goto end;
}
dest=open(destfn,O_CREAT|O_TRUNC|O_BINARY|O_WRONLY,S_IWRITE);
if (dest==-1) {
        sprintf(linebuf,"Unable to open '%s': ",destfn);
        perror(linebuf);
        goto end;
}

/* set default file range and check to make sure tha ranges are ok */
fstwvlen=400; fstwvl_dec=2; fendwvlen=800; fendwvl_dec=0;
if (startwvlen<fstwvlen) {
        printf("\nERROR: Starting wavelength to small.",fstwvlen);
        goto end;
}
if (endwvlen>fendwvlen) {
        printf("\nERROR: Ending Wavelength to large.",fendwvlen);
        goto end;
}

_dos_gettime(&time1);
curwvlen=startwvlen;
cwvlen_dec=0;
for (;;) {    /* forever */
    if (exitflag) { printf("\n ** Aborted **\n"); goto end; }
    lseek(source,0L,SEEK_SET);
    for (x=0; x<4; x++)
         if (readline(source,linebuf)) goto end;

    /* move file position to next line we want to read */
    if (cwvlen_dec==0) cwvlen_val=0;
    else if (cwvlen_dec==33) cwvlen_val=1;
    else if (cwvlen_dec==67) cwvlen_val=2;
    lseek(source,(long)((((curwvlen-fstwvlen)*3)+cwvlen_val)*24)
                                    +(fstwvl_dec*24),SEEK_CUR);
    printf("Wavelength: %d.%02d\r",curwvlen,cwvlen_dec);

    /* process current line data */
    if (readline(source,linebuf)) goto end;
    sscanf(linebuf," %*d %*c %*d %s",absorbance);
    sprintf(linebuf," %d.%02d %s",curwvlen,cwvlen_dec,absorbance);
    if (saveline(dest,linebuf,strlen(linebuf))) goto end;
```

```
            for (x=1; x<samplecount; x++) {
                lseek(source,(long)(((fendwvlen-curwvlen)*3)-cwvlen_val)*24
                                                +(fendwvl_dec*24),SEEK_CUR);
                do { if (readline(source,linebuf)) goto end;
                } while(linebuf[0]!='S');

                lseek(source,(long)((((curwvlen-fstwvlen)*3)+cwvlen_val)*24)
                                                +(fstwvl_dec*24),SEEK_CUR);
                if (readline(source,linebuf)) goto end;
                sscanf(linebuf," %*d %*c %*d %s",absorbance);
                sprintf(linebuf," %s",absorbance);
                if (saveline(dest,linebuf,strlen(linebuf))) goto end;
            }
            sprintf(linebuf,"\r\n");
            if (saveline(dest,linebuf,2)) goto end;
            if (curwvlen==endwvlen) goto complete;
            if (incwvlen==2) {
                if (cwvlen_dec==0) cwvlen_dec=33;
                else if (cwvlen_dec==33) cwvlen_dec=67;
                else if (cwvlen_dec==67) { cwvlen_dec=0; curwvlen++; }
            } else curwvlen++;
        }

    complete:
        if (writeline(dest)) goto end;
        _dos_gettime(&time2);
        m=(int)time2.minute-time1.minute;
        s=(int)time2.second-time1.second;
        if (s<0) { s=s+60; m--; }
        printf("Elasped Time = %02d:%02d\r\n",m,s);
    end:
        if (dest) close(dest);
        if (source) close(source);

        return 0;
}

/* end of file 'matrix.c' */
```

Vincent K. Lee

Lowell High School

August 6, 1992

Letterman Army Institute of Research

Mentor - Victor W. MacDonald, Ph.D.

Mechanisms of Hypertension from Hemoglobin-based Solutions

ABSTRACT

We examined the effects of hemoglobin-based solutions as blood substitutes and resuscitation fluids. Swine were subjected to hemorrhagic shock and then resuscitated with cell-free hemoglobin-based resuscitation fluids. Isolated rabbit hearts were perfused with hemoglobin-based solutions to test the mechanisms of hypertension observed in resuscitated swine. Hemoglobin's affinity for nitric oxide appears to be the primary cause of hypertension.

Mechanisms of Hypertension from

Hemoglobin-based Solutions

VINCENT K. LEE

The idea of a red cell substitute has been around for more than a century (1). The first reports of using hemoglobin (Hb) as a blood substitute date back to 1868 (1). Since that time, scientists and researchers have been scrambling to find a safe blood substitute.

The need for blood substitutes has intensified in recent years (2). Blood substitutes could relieve the limited supplies of blood available for emergency blood transfusion during a major disaster. Hemoglobin-based solutions used as blood substitutes would eliminate the problem of matching blood types during blood transfusion. Blood substitutes are being tested as potential resuscitation fluids to revive traumatized patients in situations in which access to uncompromised whole blood supplies are limited or absent. This oxygen-supplying blood substitute could save lives that might otherwise be lost.

Many successful experiments have been done to demonstrate the efficacy of hemoglobin-based solutions as possible blood substitutes (3,4). However, many safety issues remain to be resolved before this material can become a product. Hemoglobin-based

solutions have been associated with toxic side effects after being injected into patients (5,6). Vascular reactivity, leading to constriction of blood vessels, is of primary concern. Systemic vasoconstriction may cause hypertension that could inflict permanent and severe damage to the body.

The purpose of the isolated perfused heart experiment in rabbits is to examine the mechanisms whereby different types of hemoglobin-based solutions induce coronary vasoconstriction. Nitric oxide (NO) is a gas and a normal component of vascular control mechanisms that dilates blood vessels. However, hemoglobin has a high affinity for NO, and can cause vasoconstriction by interfering with normal NO-mediated vasodilation (7). Hemoglobin administration may also induce production of harmful oxygen-free radicals, which can damage tissues by attacking cell membrane lipids.

The objective of the hemorrhaged pig experiment is to determine the capabilities and hemodynamic toxicities of various hemoglobin-based solutions as resuscitation fluids. We are testing to see if hypertension exists after resuscitating a water-deprived swine with hemoglobin-based solutions.

This report will provide an overview of the testing done in the Transfusion Physiology Lab of the Blood Research Division at Letterman Army Institute of Research in San Francisco to define the potential systemic and cardiovascular toxicity of hemoglobin-based blood substitutes.

MATERIALS AND METHODS

Stroma-free hemoglobin and ααHb, formulated in Ringer's acetate, were prepared in a sterile hemoglobin production facility according to previously described methods (8).

Sterile bottles of human serum albumin in Ringer's acetate, prepared from commercial salt-poor albumin, concentrated salts, and water for injection, were used as control solutions.

Ringer's lactate was purchased from approved medical supply houses.

A. Isolated Perfused Rabbit Heart

Eight male New Zealand white rabbits, weighing 2.4 to 3.0 kg, were acquired from a commercial breeder. Animals were anesthetized with 3-1/2 cc of ketamine/Rompin/acepromazine. The thorax was opened, the heart removed immediately, and attached to an aortic cannula as previously described (9). The apparatus is shown in Figure 1. The heart was perfused with a modified Krebs-Henseleit bicarbonate (KH) buffer with a pH of 7.41. The buffer, at 37°C, was pumped through a filter. Acetylcholine and hemoglobin-based solutions were infused through a valve immediately after the filter, mixing into the buffer. The buffer then continued into an oxygenator consisting of a closed cylinder in which 25 feet of Silastic tubing (0.058 inch ID X 0.077 inch OD) was wrapped around a temperature-controlled central core. Gas mixtures (5% CO_2 plus 95% O_2) were introduced into the outer chamber around the Silastic tubing to equilibrate with the buffer, which then flowed to the heart.

A ventricular drain was created at the apex of the left ventricle to minimize ventricular filling by way of the Thebesian circulation. A

saline-filled latex balloon was inserted ~~and secured~~ into the left ventricle by an incision in the left atrium. The balloon pressure was monitored by a pressure transducer (P23XL, Gould, Cleveland, OH) and recorded on a strip-chart recorder (2400S, Gould, Cleveland, OH). The coronary venous outflow was monitored via a pulmonary artery cannula. A reservoir of 37°C KH buffer was raised under the heart until the ventricles were fully immersed.

Acetylcholine dose-response curves were generated by monitoring aortic pressure and titrating the drug in doses of: .2μM, .5μM, 1μM, 2μM, 4μM, 6μM, 8μM, and 10μM. The stability of the aortic pressure determined when the next dose of acetylcholine was administered. A set of baseline acetylcholine concentration versus aortic pressure measurements was established. After a brief period of 5 to 10 minutes of circulating pure buffer, the hemoglobin-based solution was introduced. Another acetylcholine titration curve was determined in the presence of the hemoglobin-based solution. After another period of circulating pure buffer through the system, a recovery dose-response curve for acetylcholine was determined.

At the end of the experiment, the ~~atria and the right~~ ventricle were trimmed. The left ventricle was opened and lightly blotted. A wet weight was immediately taken and a dry weight was taken after drying overnight at 110°C.

The left ventricular developed pressure (LVDP), derivative of LVDP, coronary inflow PO_2, and coronary outflow PO_2 were monitored and recorded on a strip chart recorder. Oxygen consumption was determined by measuring the difference between the content of perfusate (buffer) entering and exiting the coronary circulation, using in-line Clarke-type oxygen electrodes (Instech, Horsham, PA). All the collected data were entered into a computer spreadsheet (RS1, BBN Software Products Corp., Cambridge, MA) (9).

B. Hemorrhaged Pigs

Six 8- to 12- week old female swine weighing 13 to 18 kg, were acquired from a commercial breeder. Animals were surgically prepared by methods previously described (8). The animal preparation is shown in Figure 2. Each animal was fasted overnight.

It was given anesthesia with a mixture of isoflurane, nitrous oxide, and oxygen. A carotid artery catheter (1 mm ID) was placed in one carotid artery through a midline neck incision. A central venous catheter (1 mm ID) was inserted into one internal jugular vein, and a Swan-Ganz catheter (Pentacath, Viggo-Spectramed, Oxnard, CA) was inserted through the other internal jugular vein into the pulmonary artery. The incision was closed after the catheters were brought through the skin on the dorsal surface of the neck. A midline laparotomy was performed, following the same procedure, to remove the spleen (8,10).

Finally, an aortic catheter (2 mm ID) was introduced, and a left renal artery flow probe (6R, Transonic Systems, Inc., Ithaca, NY) was attached through a left retroperitoneal incision and brought through the skin on the dorsal midline. Velcro pouches were sewn to the skin to hold the external ends of the catheters. The catheters were flushed with heparinized saline and capped. Anesthesia was discontinued, and the animal was moved to a transfer cage, where it was observed until it awoke and then it was returned to the animal care facility (8,10).

Two days before the hemorrhage, the swine was transferred to a metabolic cage. The swine was water-deprived for two days, and fasted for 12 hours before the hemorrhage. The animal was weighed daily. Blood samples were drawn daily for blood chemistries and urine samples were collected for volume and creatinine concentration.

The swine was moved to a laboratory in its metabolic cage on the day of hemorrhage. The catheters and the flow probe were connected to pressure transducers and recording machines. Two sets of baseline blood samples were taken 15 min apart after the readings of the aortic, pulmonary artery, and central venous pressures stabilized. When hemorrhage began, aortic blood (25 ml/kg) was withdrawn by a syringe pump (Model 22, Harvard Apparatus, South Natick, MA) over the next hour at 15 min intervals. Blood samples were taken at each of these 15 min intervals. All blood samples were taken for blood chemistries and the pH, PCO_2, and PO_2, using the blood gas analyzer (Model 170 pH/Blood Gas Analyzer, Corning, Medfield, MA). Withdrawn blood was stored in CPDA-1 at room temperature for later reinfusion.

One hour after hemorrhage began, the swine was given the resuscitation fluid through the central venous catheter. Blood samples were taken at five more time points following the resuscitation. Fifteen minutes after the last blood sample, the swine was transfused with its own filtered blood, which had previously been withdrawn. A final set of blood samples were obtained. The catheters were finally flushed with heparin and placed back into the dorsal pouches, along with the flow probe wires. The swine was given free access to food and water.

Pressure transducers (P23XL, Gould, Cleveland,OH), connected to the catheters, measured the aortic, central venous, and pulmonary artery pressures. Outputs were recorded on a strip-chart recorder (3800S, Gould, Cleveland, OH). Cardiac output was measured in triplicate by thermodilution with the Swan-Ganz catheter and a cardiac output computer (COM-1, American Edwards Laboratory, Irving, CA). The ultrasonic flow probe, connected to the flow meter (Model 201, Transonic Systems, Inc., Ithaca, NY), measured the left renal artery flow. The filtered urine, collected from the bottom of the metabolic cage was measured for volume in a graduated cylinder. All

data were then entered into a computer spreadsheet (RS1, BBN Software Products Corp., Cambridge, MA).

The next day, the swine was taken to the necropsy room. It was anesthetized with sodium pentobarbital and urine specimens were collected from the dissected right ureter. Euthanasia was performed with an overdose of sodium pentobarbital. Body tissues and cavities were examined and abnormalities were recorded. Sections of various organs, including the eye, heart, lung, liver, kidney, and cerebrum, were removed and stored in 10% neutral buffered formalin and tissues were taken and prepared for light microscopic examination. All abnormalities were recorded, and then all tissues were graded for all lesions (8,10).

RESULTS

A. Isolated Perfused Rabbit Heart

It has been shown that aortic pressure increases as acetylcholine is administered to hearts in progressively increasing

concentrations (Fig. 3). When hearts are treated with ααHb, sensitivity to acetylcholine increases, indicated by a shift to the left of the dose-response curve. This increased sensitivity does not return completely to normal after ααHb is removed unless deferoxamine is also present as shown in Figure 4.

Four hearts treated with cyan-met Hb (CNmetHb) showed a slight shift toward the left in the dose-response curve (Fig. 5). However, when CNmetHb was removed the recovery curve indicated even higher sensitivity to the drug than the other two curves.

Two hearts with the CNmetHb and deferoxamine had entirely different results (Fig. 6). The control, experimental, and recovery values were so close that no differences were observed.

One heart was administered nitroglycerin, a nitrovasodilator drug (Fig. 7). Sensitivity to acetylcholine appeared to be unaffected by this treatment.

Another heart received ααHb with 2-Mercaptopropionyl glycerin, another scavenger of oxygen-free radicals that, unlike deferoxamine, does not bind free iron. This compound did not affect the increased sensitivity to acetylcholine due to ααHb (Fig. 8).

Renée M. Ward

Tamalpais High School - Pomona College

August 5, 1992

Letterman Army Institute of Research

Mentors - Gerald L. Moore, Ph.D. and Mary Edith Moore

Development of New Methods for Post-Thaw
Red Blood Cell Preservation

Abstract

Fresh human blood was collected in CPDA-1, the red blood cells (RBCs) were frozen by the Valeri high glycerol technique and stored at -80°C. Later, the RBCs were thawed, washed, and an additive solution developed at the Letterman Army Institute of Research (LAIR) was added. The RBCs were stored at 4°C for 14 or 21 days and assayed for ATP, 2,3-DPG, glucose, pH, P50, lysis, crenation, sodium, potassium, and osmolarity. The RBCs were tagged with radioactive chromium, and infused into the original blood donors. Further assaying in a gamma counter determined the percentage of RBCs retained by the donors 24 hours later. Research to date looks promising, but further studies are needed to determine if this additive solution can be used for extending the shelf life of thawed RBCs to 21 days.

Development of New Methods for Post-Thaw

Red Blood Cell Preservation

Renée M. Ward

The freezing of red blood cells (RBCs) could be an ideal way for blood banks to store rare blood types and stock blood for use in treating casualties of war or local emergencies, such as an airplane crash. However, cryopreservation of RBCs is currently impractical for wide-scale use because of the expense of freezing blood, which is four times that of storing fresh blood, and the limited 24-hour shelf life after thawing (1). The goal of our research is to make cryopreservation more practical by extending the 24-hour post-thaw life of the RBCs to 14, and perhaps even 21, days.

One of the current problems with frozen red blood cells is transportation. With only 24 hours of post-thaw shelf life, RBCs must currently be kept at -80°C while being transported (2). This requires special generators in addition to extra time and money. Two weeks of RBC viability after thawing would not only give blood banks enough time for transportation, but would vastly increase the now limited shelf life. This improved accessibility and extended viability could save the lives of people with rare blood types. In addition, these improvements would benefit the military, which has several frozen blood banks around the world intended for use during the initial stages of an armed conflict (3). Presently, these blood reserves have limited practicality because the thawed RBCs must reach the Deployable Medical System at the site of combat and be infused within 24 hours.

Our research, however, hopes to improve the effectiveness of these blood banks by providing 14 to 21 days of post-thaw shelf life.

Another benefit of our research is that it has the capability of making the nation's blood supplies safer (2). Currently in the United States, the annual number of clinical cases of post-transfusion infection with the human immunodeficiency virus (HIV) is estimated at 70, while the estimated risk of contracting HIV from one unit of transfused blood is 0.0007% (4). However, some diseases, such as HIV, cytomegalovirus, and human T-lymphotropic retrovirus, are screened by identifying antibodies to these viruses. Several months can pass after the initial exposure before an antibody response is evident, and thus infected blood with undetected antibodies passes screening each year (4). Because most donors give blood repeatedly, their RBCs could be frozen until the next occasion they gave blood, at which time it would be screened. If any diseases with long incubation periods, such as HIV, appeared, the frozen blood would be disposed of, with the possibility that it, too, was contaminated.

The potential for frozen RBCs has been overlooked due to its expense and limited post-thaw shelf life. However, the cost is steadily decreasing as new and better freezing techniques are developed (2); and, with an extended shelf life, the motivation to find cheaper methods for freezing RBCs will be even greater. This study hopes to shed light on the benefits of cryopreservation of RBCs, allowing for its use in practicality and efficacy.

Materials and Methods

Freezing

To obtain the frozen RBCs needed for our research, whole blood was collected in oversized 800 mL bags containing 63 mL of CPDA-1 (manufactured by Baxter Healthcare Corporation, Deerfield, IL). One sample was taken from the bag at this time for use in a P50 assay.

The whole blood was then centrifuged, in a Beckman J6-HC (Fullerton, CA), for 10 min at 3532 x g (5) at room temperature to separate the plasma from the RBCs. The plasma was then squeezed into a connecting bag with a plasma press, clamped off and saved for use in micro-Drabkins, sodium, potassium, and osmolarity assays. A sample of the red blood cells was used for hematocrit, morphology, hemoglobin, blood pH, P50, and fluorometric assays (See assay section of materials and methods for procedures.)

To prepare the packed RBCs for freezing, we added a total of 400 mL of Fenwal Glycerolyte 57 solution (Deerfield, IL). The top of each glycerol bottle was first swabbed with rubbing alcohol and then punctured with a plasma transfer set tube (Fenwal #4C2243; Deerfield, IL) and a vent tube (Becton Dickinson #5200 19G1 1/2 TW; Rutherford, NJ). The RBC bag was placed on an Eberbach shaker machine (Ann Arbor, MI) and the bottle of glycerol inverted. As the RBCs were gently being shaken, 50 mL of glycerol were added. After a 5 min rest with the shaker turned off, an additional 50 mL of glycerol were added (shaker on). After 2 min of rest (shaker off), the remaining glycerol was added (while being mixed by hand). Special care was taken to shut off the flow of

glycerol immediately before the last few drops were added. This last step prevents air from getting into the bag.

Next, we centrifuged the glycerolized RBCs at 1250 x g (5) for 10 min at room temperature. Once again, we used a plasma press to squeeze off the supernatant, excess glycerol, into a connected bag, clamped off the bag, and discarded it. The RBCs were then placed in a sealed freezer bag and cardboard box and frozen at -80°C.

Thawing and Washing

Each bag of frozen RBCs was placed in a Precision Coliform Incubator Bath (Fisher Scientific; Santa Clara, CA), and heated to a temperature between 37°C and 42°C. The bag remained in the wash bath until the blood thawed and reached 37°C; this takes approximately 35 min.

A 2000 mL bag of 0.2% dextrose and 0.9% sodium chloride processing solution (Fenwal; Deerfield, IL), a 150 mL bag of 12% sodium chloride processing solution (Fenwal; Deerfield, IL), a 5 L waste bag (Haemonetics; Braintree, MA), and a 600 mL transfer pack (Fenwal; Deerfield, IL) were then attached to a wash set for the Haemonetics Blood Processor 115 (Braintree, MA). The RBCs, saline (12% sodium chloride) and wash solution (0.2% dextrose and 0.9% sodium chloride) should be positioned according to the method of Valeri (5). The RBCs were then mixed on the platform of the Haemonetics Blood Processor 115 while 50 mL of the 12% saline were added. Two minutes of rest followed with the mixer off. (If at this time any pooling, dark spots, occurs in the RBCs, the cells must be mixed by hand.) With the mixer on, 100 mL of wash

solution were added, 2 min rest (mixer off), 150 mL wash solution added (mixer on), and 2 min rest (mixer off). The centrifuge in the cell washer was then turned on and the dilute RBCs added. The flow rate of the blood into the bowl should be approximately 75 mL per minute, and the bowl should not fill in less than 5 min. When effluent appeared in the waste line, the rest of the wash solution was added. If the waste solution appears murky in the tubing, the RBC bag should be lowered to prevent the RBCs from spilling over into the waste bag. After 1000-1200 mL of wash solution have been added, the effluent is normally clear, registering less than 150 mg % free hemoglobin. This can be evaluated with a Free Hemoglobin Visual Comparator (Haemonetics Corporation; Braintree, MA). If more free hemoglobin is present, the RBCs need further washing with wash solution. Once all of the wash solution was added, the centrifuge was stopped and the RBCs were forced out of the bowl and siphoned into the 600 mL transfer pack (Fenwal; Deerfield, IL). The tubing between the bowl and transfer pack was clamped off.

The washed RBCs were centrifuged at 1500 x g, room temperature for 10 min to remove excess wash solution. The supernatant was squeezed off with a plasma press and discarded. One hundred milliliters of an additive solution developed at LAIR was then added to the RBCs and mixed by hand. A sample was taken for assaying and the remaining RBCs were stored at 4°C.

Assays

The following assays were conducted on the same day (day zero) that the RBCs were thawed and washed.

Osmolarity Assay (Appendix I)

We assayed 0.3 mL of plasma from each sample in triplicate on an Osmometer Model 3DII (Advanced Instruments Inc., Needham Heights, MA.) (6).

P50 Assay (Appendix II)

In a cuvette, we prepared a buffer solution of 5 mL Hemox solution and 20 μL of anti-foam. We covered the cuvette with parafilm and then warmed it in a heating block to 38°C. Once the buffer reached its desired temperature, we poured it into the sample chamber of a Model B Hemox-Analyzer manufactured by TCS Medical Products Company (Huntingdon Valley, PA). We added 50 μL of sample to the buffer solution. The rest of the P50 assay was done according to the Operation Manual for the Hemox-Analyzer (7).

Hematocrit (Appendix III)

Two heparinized hematocrit tubes were filled with sample by capillary action. They were centrifuged in a micro hematocrit IEC MB centrifuge (Needham Heights, MA) for 5 min, and then read with an IEC International Micro-Capillary Reader (Needham Heights, MA).

Morphology (Appendix IV)

We fixed a sample of RBCs with a solution containing 2% glutaraldehyde, 0.1 mol/L Na-cacodylate HCl (pH 7.2), and 0.1 mol/L sucrose (8). A 1:2 dilution of sample to fixative was made. The sample can now be stored at 4°C until the cells are viewed or counted under the microscope.

To do the morphology, we made a 1:120 dilution of sample to diluent (8.5g sodium chloride and 0.1g sodium azide per liter of distilled water) for easier cell counting. This dilution is based on a hematocrit of 40, higher hematocrits need a greater dilution factor, lower hematocrits require less dilution. Twenty microliters of the dilute fixed cells were then placed in a hemocytometer and viewed through a Universal Zeiss Microscope (Germany) with a blue filter and a 40-power objective. Three slide pictures of each sample were taken with an attached Nikon 2000 camera (Tokyo, Japan). Each slide was of a different set of sixteen squares on the hemocytometer.

Later, the slides were viewed and the RBCs counted and categorized according to the level of degradation of the RBCs (Fig. 1) (9). Each category was then placed in the following equation to rate the RBCs (8):

$$\frac{(A\ 1100/E) + (B\ 800/E) + (C\ 400/E) + (D\ 100/E)}{11}$$

Micro-Drabkins Assay (Appendix V)

An aliquot of sample was centrifuged, and the supernatant removed. We determined the amount of hemoglobin in the supernatant with a Cobas Fara

(Roche Diagnostic Systems; Montclair, NJ). The temperature was set at 25°C, wavelength at 540 nm, and 200 μL of Drabkins solution were used as a reagent. Seven readings were taken, one every 60 seconds (10).

Sodium and Potassium Assay (Appendix VI)

Small aliquots of sample were centrifuged and the supernatant was drawn off. The supernatant was then analyzed in a Corning 480 Flame Photometer (Medfield, MA) with Corning Lithium Internal Standard 3000 (Medfield, MA). We used the method described in the instruction manual (11).

Hemoglobin Assay (Appendix VII)

Prepared in triplicate, 20 μL of sample were added to 5 mL of Drabkins reagent and gently mixed. A Beckman Spectrophotometer DU-62 at 540 nm (Fullerton, CA) was zeroed with a blank containing Drabkins reagent. A standard of cyanmethemoglobin at known concentration was prepared in a corresponding cuvette. Both the standard and sample were read in the spectrophotometer. (Readings for the sample and standard should be with in 0.005 of each other, if not, repeat assay) (12).

Blood pH (Appendix VIII)

In a Corning 170 pH/Blood Gas Analyzer (Medfield, MA), the temperature was set at 37°C, the gas cylinder pressure at 300 psi, the regulator pressure to 3.5-4.5 psi, and the barometric pressure entered. A sample was put into the sample port of the pH/Blood Gas analyzer via syringe. The procedure followed the guidelines in the instruction manual (13).

Fluorometric Assays

Due to time constraints, the fluorometric assays were not conducted during my tenure at LAIR. These assays determine the amount of adenosine triphosphate, glucose, and 2,3-diphosphoglycerate in the RBCs. According to the protocol of the experiment, this procedure is done using the Moore technique (14).

<u>Infuse Patient</u>

After 14 or 21 days, we took 20 mL of sample and added 20 μL of radioactive sodium chromate. The sample was then allowed to sit for 20 min at room temperature with periodic mixing. At the end of 20 min, we added two doses of 30 mL 0.9% saline solution. Next, we centrifuged the sample for 10 min at 1000 x g. After centrifuging the sample, a spinal needle was inserted into the bottle to draw off the supernatant. We weighed the sample, measured its radioactivity with a dosimeter, put it in a syringe, and injected it into the original donor. Five minutes later we withdrew a 5 mL sample of the donor's blood and repeated this step five times, once every 2.5 min. Twenty-four hours later, another 5 mL sample was withdrawn from the donor.

One milliliter of each sample was then diluted with 1 mL of water. These dilutions were made in triplicate for each of the seven samples, and then placed in a gamma counter for a read-out on the radioactivity of each sample.

<u>Assays</u>

After 14 or 21 days, the assays performed on day zero were repeated.

Results

On the P50 assay I ran on fresh blood, the oxygen-carrying capacity, 25 mm Hg, was lower than that of average fresh blood, 27 mm Hg (15). This confirms our average P50 at zero day of 26.5 mm Hg. For the P50 assays, the temperature was kept at 37°C and the buffer at pH 7.4.

The results from the micro-Drabkins assay had a total average of 0.0696 g of hemoglobin per dL of supernatant. The average osmolarity was 304 mOsm/kg supernatant. The total average hematocrit on day zero, the day of thawing, was 67.9. On day zero the total average morphology index was 100. After 21 days, the total average morphology index dropped to 82.95. The total average blood pH at zero day was 7.028. For the hemoglobin assay at zero day, the total average was 22.9298 Hb/dL.

After freezing, the level of sodium (Na^+) in the supernatant returned to 97.4% of its pre-frozen level and potassium (K^+) in the supernatant returned to 49.9% of its pre-frozen level. After 14 days, the Na^+ concentration decreased 30.6%, from 170.9 mM Na^+/L supernatant before freezing to 118.6 mM Na^+/L supernatant at 14 day post-thaw. The K^+ level increased 1331.9%, from 3.54 mM K^+/L supernatant before freezing to 47.2 mM K^+/L supernatant at 14 day post-thaw. After 21 days, the average concentration of Na^+ in the supernatant decreased 27.9%, from 172.[illegible] mM Na^+/L supernatant before freezing to 124.6 mM Na^+/L supernatant at 21 day post thaw. After 21 days, the average concentration of K^+ in the supernatant increased 1449.6% from 3.[illegible] mM K^+/L supernatant before freezing to 53.2 mM K^+/L supernatant at 21 day post thaw.

Discussion

The results of the different assays indicate the efficacy of the additive solution in preservation of the RBCs. Some of the assays are also used to ensure that the additive solution meets the regulations set up by the Food and Drug Administration (FDA).

On the single P50 assay I ran on fresh blood, the results were insignificant. But, if that blood had been frozen later that day and eventually thawed, the results from the assay I performed would have been used to compare the oxygen-carrying capacity of the thawed RBCs to their oxygen-carrying capacity before freezing.

The P50 data at zero day, however, indicated that the freezing and thawing process had no impact on the oxygen-carrying capacity of the RBCs, because the results were the same as those for normal fresh blood.

The results of the hemoglobin assay on RBCs would typically be used as a correction factor for the ATP and 2,3-DPG assays; however, because these assays are not documented in this paper, the results of the hemoglobin assay have not been used.

The purpose of the micro-Drabkins assay is to determine the amount of lysis that occurs in the RBCs. If the RBCs are ruptured, hemoglobin is released. To ensure that the majority of the RBCs are in good condition, the FDA maintains that the hemoglobin present in the supernatant is less than 1 g %. The average amount of hemoglobin per dL of supernatant in our study was 0.0690 g/dL, much less than FDA requirements

The average osmolarity of 304 mOsm/kg supernatant indicates the osmotic pressure of a molal concentration of an ion in the solvent. Normal osmolarity for

fresh plasma is 320 mOsm/kg (Personal communication, G. Moore, Ph.D., 4 Aug. 92). Thus, this data indicates relatively normal osmotic pressure in the plasma before the freezing procedure took place.

By rating the RBCs via the morphology index, we are able to determine the condition of the RBCs. A high morphology index indicates that only a small percentage of the RBCs are crenating. On the day of thawing, the morphology index was 100; all of the RBCs were in the healthy smooth disc form. Thus, although the freezing, thawing, and washing of the RBCs are traumatic processes, they had no impact on the degradation of the RBCs. The total average morphology index for 21 days was 82.95, indicating that post-thaw storage does allow for the crenation of the RBCs. However, the normal life span for a RBC is 120 days (15), and thus over the course of 21 days, a certain percentage of the RBCs would be crenating due to their normal aging process. Because the morphology index is still high at 21 days, we can infer that the additive solution is effectively minimizing deterioration of the RBC membranes.

Normal blood has a pH between 7.0 and 7.2. At zero day, the normal pH was maintained. Previous studies have shown that when a normal pH level is maintained, the RBCs retain more of their oxygen-carrying capabilities. Thus, the freezing and thawing processes did not hinder the oxygen-carrying capability of the RBCs. This is reinforced by the P50 data at zero day, in which the normal oxygen-carrying capacity of the RBCs was maintained.

The levels of sodium (Na^+) and potassium (K^+) in the blood help the body regulate water retention, and are necessary to maintain life (16). Relatively high concentrations of Na^+, approximately 140 μM/mL, and low concentrations of K^+,

4-5 μM/mL, are found in extra cellular fluids, although inside the cells, Na^+ is low, 10-20 μM/mL, and K^+ is high, 90-100 μM/mL (17). During the freezing of RBCs, K^+ leaves the cell and Na^+ enters it. After thawing, however, the concentrations of Na^+ and K^+ in the RBCs should return to normal, unless the cell membranes have been damaged (16). Thus the 2.6% difference between pre-frozen and thawed concentrations of Na^+ in the supernatant and the 50% difference between pre-frozen and thawed concentrations of K^+ in the supernatant indicate some damage occurred in the RBC membranes during freezing, thawing, and washing. After 14 days, Na^+ concentrations decreased another 46.3 mM Na^+/ L supernatant (from concentration on day of thawing). Similar results were obtained after 21 days; thus, at this point a plateau was reached, and the additive solution maintained the concentrations of Na^+ and K^+ in the supernatant.

From the clinical trials, when donors were infused with their own blood after 14 or 21 days of post-thaw storage, the average in vivo survival measurements were above the FDA required level of 75% for both 14 and 21 days. (Details will be published at a later date.)

Throughout this study, RBCs remained safe and viable. Lysis and degradation of the RBCs were kept at a minimum, and the oxygen-carrying capacity of the RBCs maintained. Thus, except for disrupting the concentrations of Na^+ and K^+, these assays determined that the freezing and thawing procedures had minimal impact on the RBCs.

Conclusion

The RBCs from this study are still being analyzed. Eventually, the compiled data will be sent to the FDA for approval. This research is, however, discovering new methods and additive solutions that will eventually extend the post-thaw shelf life of RBCs.

Acknowledgments

In appreciation for their time, patience, and encouragement, I would like to thank my mentors, Dr. and Mrs. Moore. During this summer, they have shown me that science is a living field that is constantly evolving, and that being a part of that evolution is both challenging and rewarding.

Mrs. Siefert has been an enormous help with the writing of this paper, in particular by helping me understand the format of a scientific paper.

For his explanations on how to conduct many of the assays, time, and encouragement, I would like to thank Angulo Zegna.

Many thanks to Conrad Wheeler, Ph.D. for helping me locate a computer to work on, and his assistance with the graphs and formatting of this paper.

The first three weeks of this apprenticeship were spent in Quality Assurance with LT Cima. During that time, I learned about the "behind the scenes" process that allows research to be conducted here at LAIR. LT Cima's time and encouragement were greatly appreciated, along with his understanding, which allowed me to relocate midway in this program to allow for more hands-on lab work.

I would also like to thank the entire staff here at LAIR for making me feel welcome and for answering my many questions. Your time and kindness have been appreciated.

Human Use Statement

Human Subjects participated in these studies after giving their free and informed voluntary consent. Investigators adhered to AR 70-25 and USAMRDC Reg 50-25 on the use of volunteers in research.

References

1. Chaplin H. Clinical Uses of Frozen-Stored Red Blood Cells. In: Petz LD, Swisher SN, eds. Clinical Practice of Transfusion Medicine; Second Edition. New York: Churchill Livingstone Inc., 1989:315-323.

2. Valeri CR. Blood Banking and the Use of Frozen Blood Products. New York: CRC Press, Inc., 1976:53-58;341-342;367.

3. Moore GL, Ledford ME. Additive Solutions for the 21-Day Preservation of Previously Frozen Red Blood Cells. The Journal of the US Army Medical Department 1991;Jan/Feb:14-19.

4. Nance ST, ed. Transfusion Medicine in the 1990's. Arlington: American Association of Blood Banks, 1990:223-252.

5. Valeri CR. Standard Operating Procedure For Freezing and Thawing Red Blood Cells. Boston, Massachusetts: Naval Blood Research Laboratory.

6. Advanced Instruments Inc. Service Manual for the Osmometer Model 3DII. Needham Heights, Massachusetts: 1979.

7. TCS Medical Products Company. Operation Manual for the Hemox-Analyzer. Huntingdon Valley, Pennsylvania: 1991; Model B Hemox-Analyzer.

8. Högman CF, de Verdier CH, Ericson Å, Hedlund K, Sandhagen. Studies on the Mechanism of Human Red Cell Loss of Viability during Storage at +4°C in vitro. Vox Sang 1985;48:257-268.

9. Usry RT, Moore GL, Manalo FW. Morphology of Stored, Rejuvenated Human Erythrocytes. Vox Sang 1975;28:176-183.

10. Roche Diagnostic Systems. Cobas Fara Chemistry System Method Reference Manual. Montclair, New Jersey: 1987.

11. Corning. Instruction Manual: Corning 480 Flame Photometer. Medfield, Massachusetts: 1983.

12. Beutler E. Red Cell Metabolism. 2nd ed. New York: Grune and Statton, 1975:11-12;29-32;76.

13. Corning. 170 pH/Blood Gas Analyzer Instruction Manual. Medfield, Massachusetts: 1982.

14. Moore GL, Ledford ME: Development of an optimized additive solution containing ascorbate-2-phosphate for the preservation of red cells with retention of 2,3-diphosphoglycerate. Transfusion 1985;25:319-324.

15. Hillman RS, Finch CA. Red Cell Manual. 5th ed. Philadelphia: F.A. Davis Company, 1985:1-29.

16. Giese AC. Cell Physiology. 3rd ed. Philadelphia: W.B. Saunders Company, 1968:36;310-331.

17. Weissman G, Claiborne R, eds. Cell Membranes: Biochemistry, Cell Biology and Pathology. New York: Hospital Practice Publishing Company, Inc., 1975:95-104.

Appendix I

Osmolarity before Freezing

Patient	mOsm/kg supernatant before freezing			Average (mOsm/ kg supernatant)
	sample 1	sample 2	sample 3	
340	301	305	302	303
341	311	311	312	311
342	311	312	309	311
343	307	303	303	304
344	300	303	300	301
345	312	310	313	312
346	299	303	297	300
347	300	304	303	302
358	298	300	304	301
350	316 *	301	301	301
351	302	300	307	303

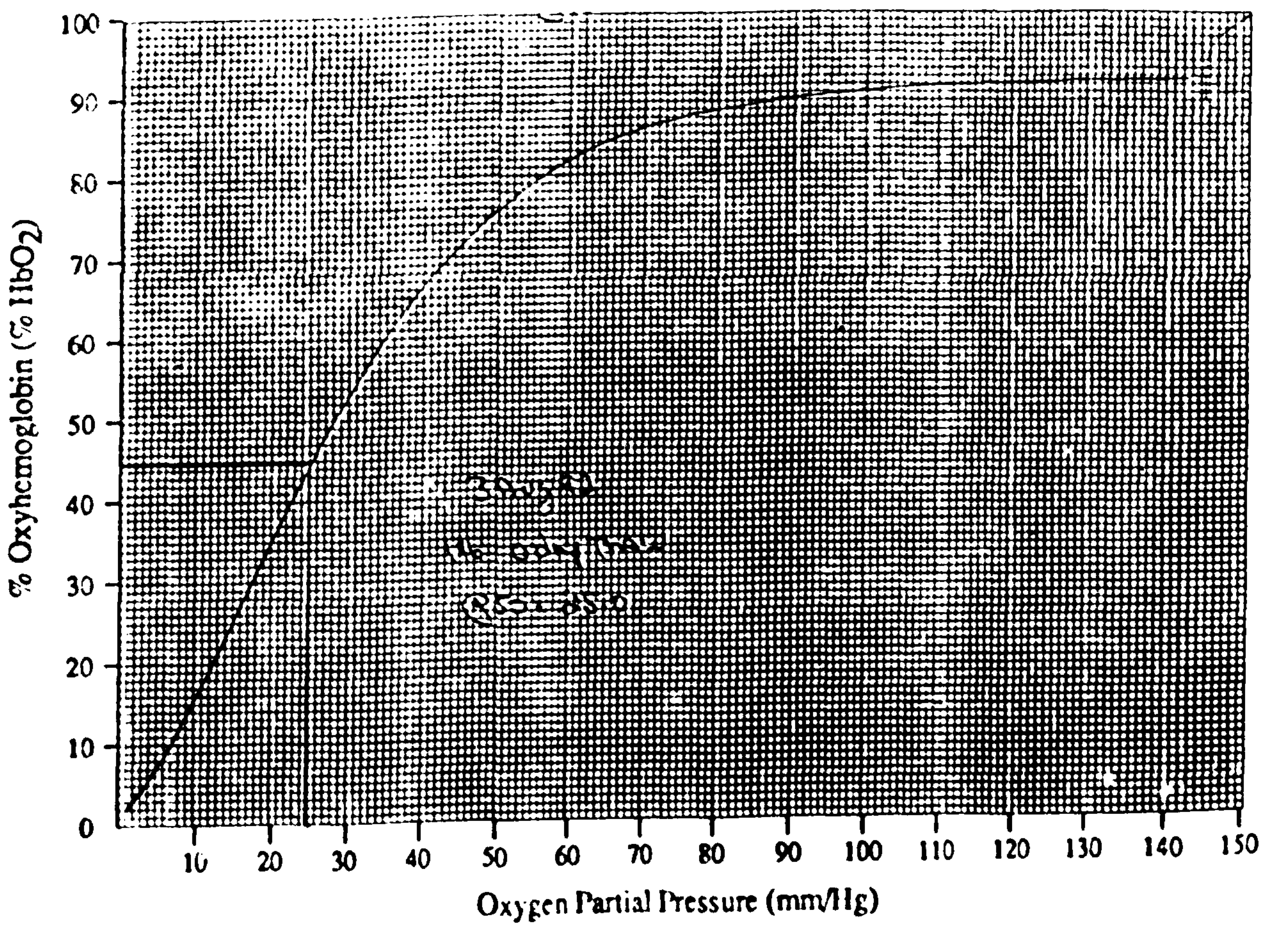
*Discarded when average calculated.

Total Osmolarity Average before freezing (mOsm/ kg supernatant)	304

Appendix II

P50 at Zero Day

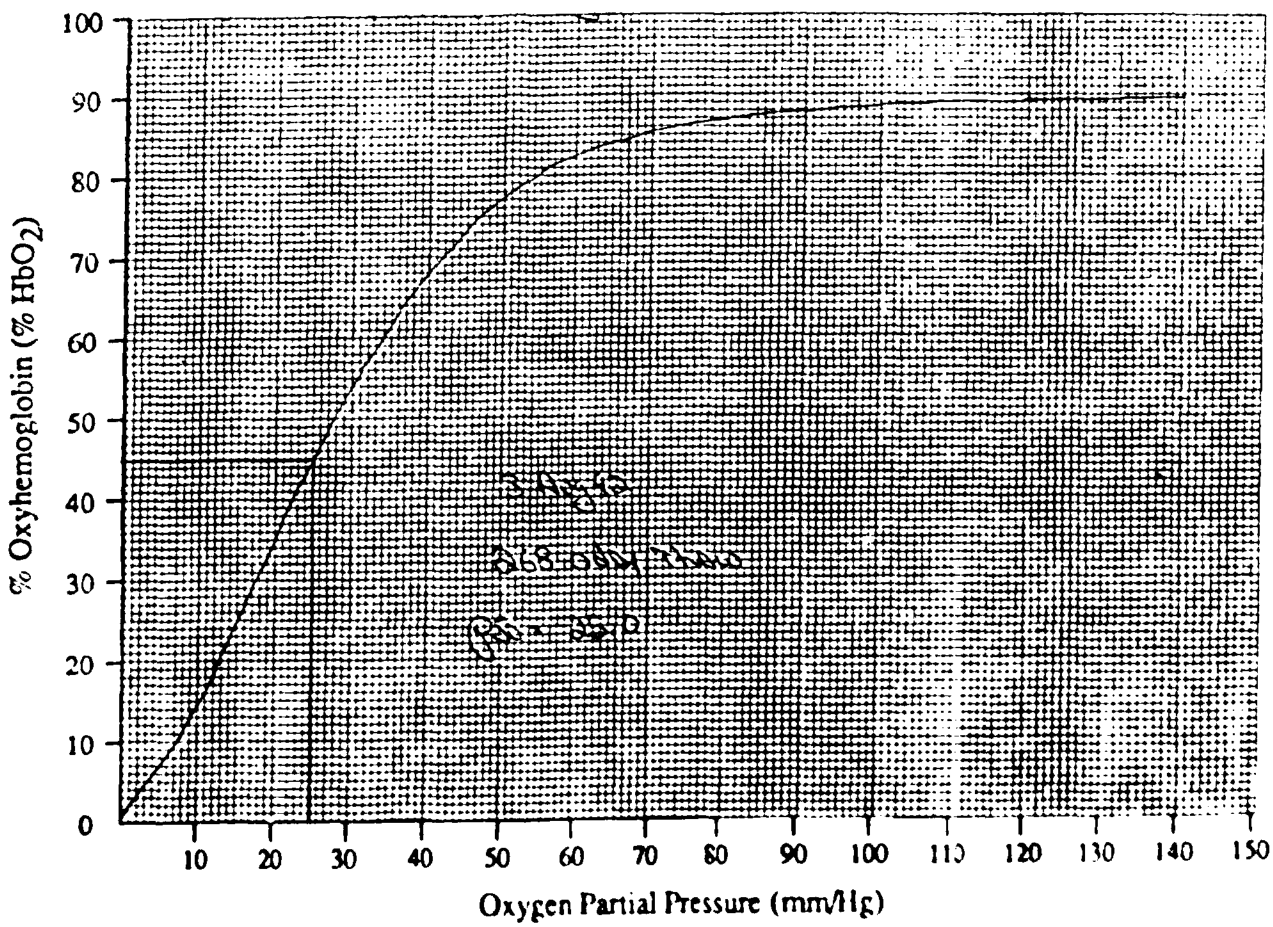
Patient:	196
Date:	3 Aug. 92
Temp.:	37.1°C
pH:	7.4
P50:	25.0 mm Hg



20--Ward

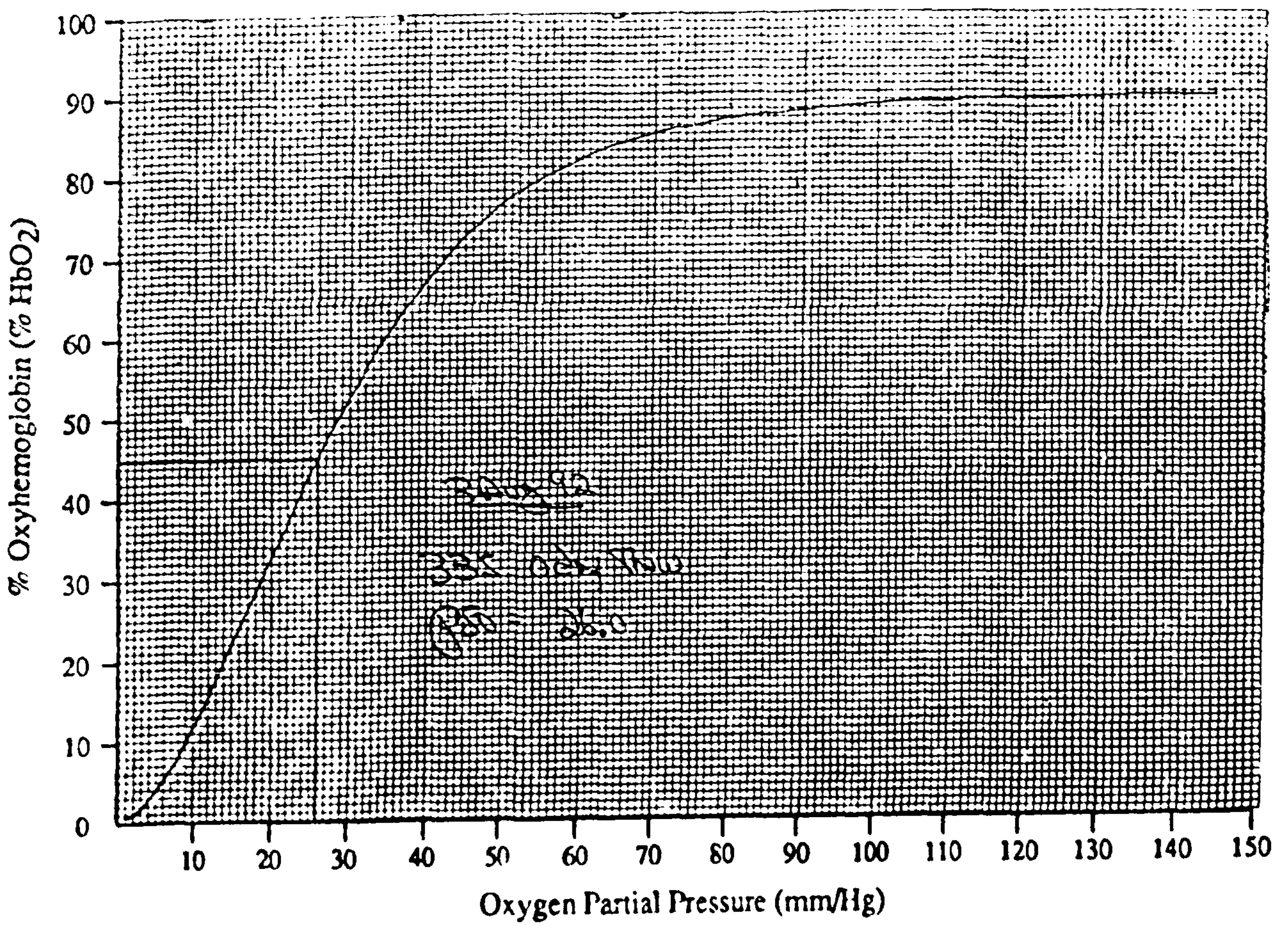
P50 at Zero Day

Patient: 268
Date: 3 Aug. 92
Temp.: 37.1°C
pH: 7.4
P50: 25.0 mm Hg



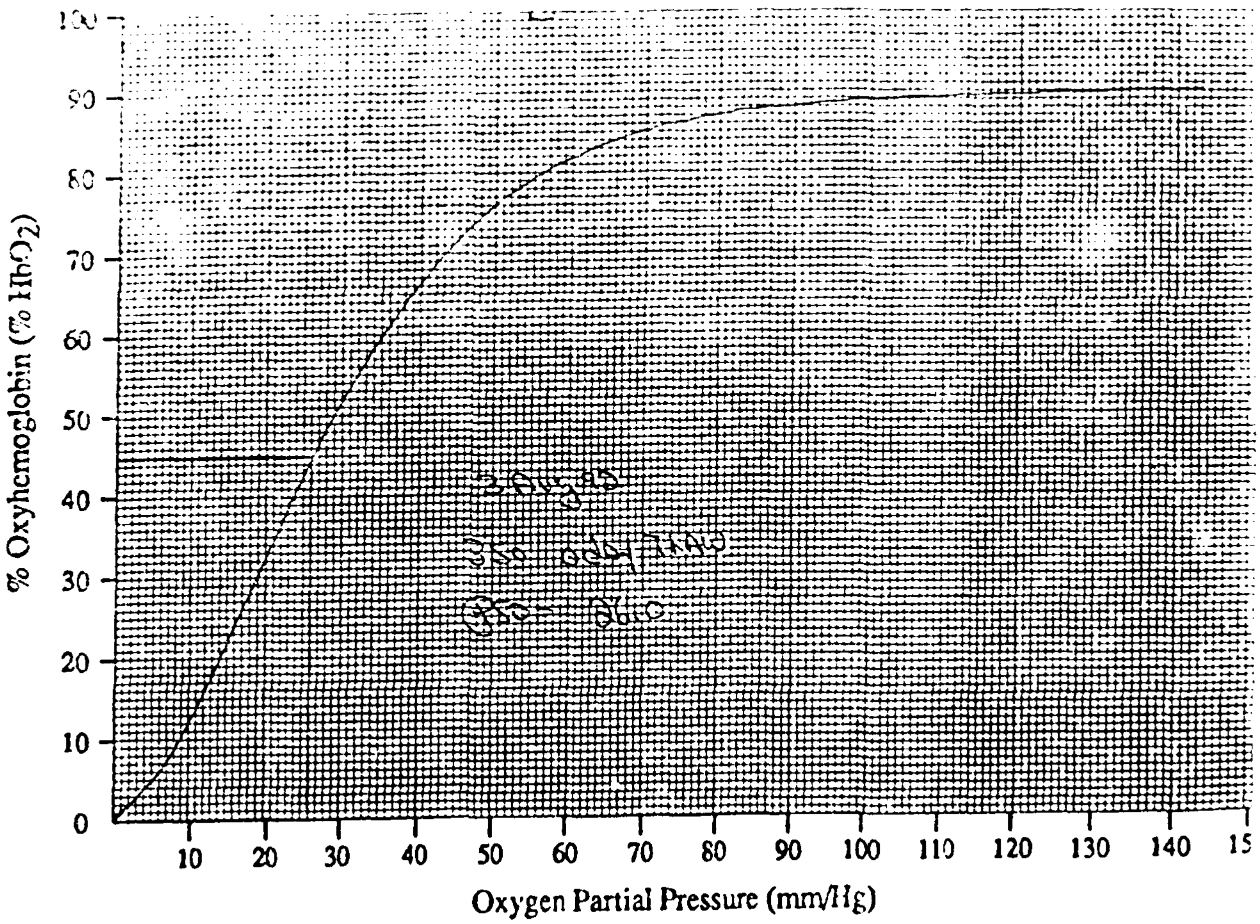
P50 at Zero Day

Patient:	335
Date:	3 Aug. 92
Temp.:	37.1°C
pH:	7.4
P50:	26.0 mm Hg



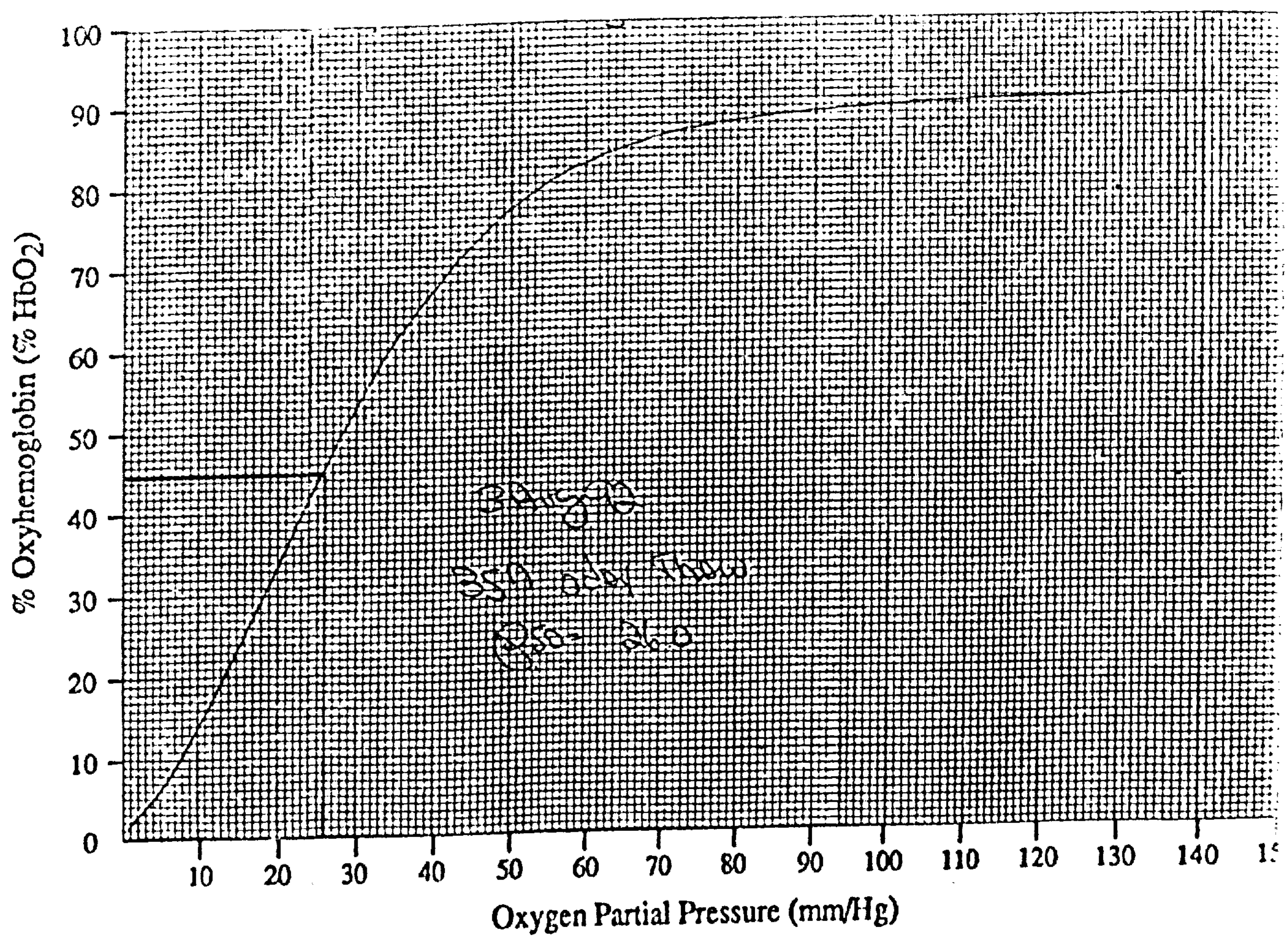
P50 at Zero Days

Patient:	350
Date:	3 Aug 92
Temp.:	37.1°C
pH:	7.4
P50:	26.0 mm Hg



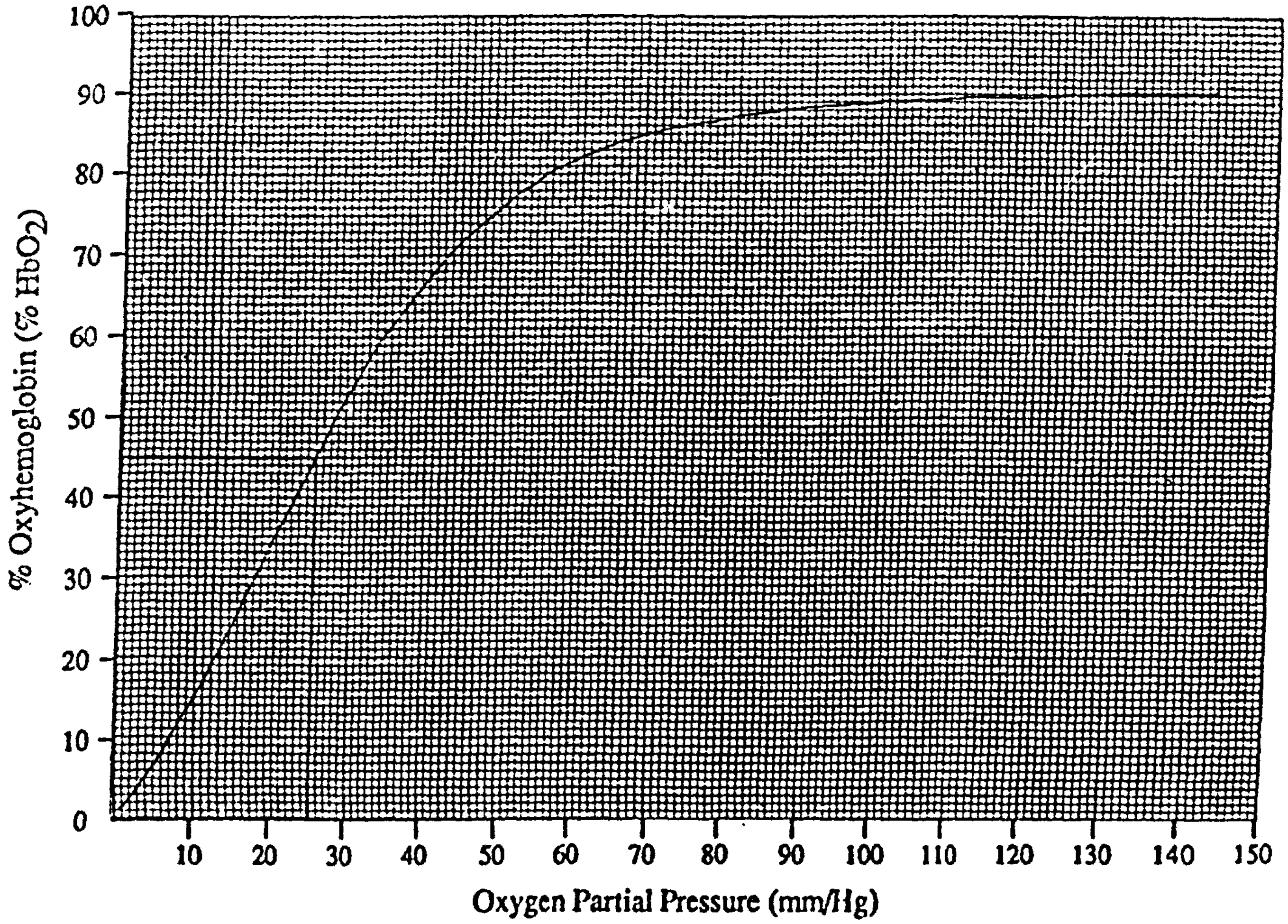
P50 at Zero Day

Patient:	357
Date:	3 Aug. 92
Temp.:	37.1°C
pH:	7.4
P50:	26.0 mm Hg



P50 on Fresh Blood before Freezing

Patient:	500
Date:	29 July 92
Temp.:	37.1°C
pH:	7.4
P50:	25.0 mm Hg



Appendix III

Hematocrits at Zero Day

Patient	Hematocrits at Zero Day		Average Hematocrit
	sample 1	sample 2	
196	71.0	71.0	71.0
268	68.0	68.0	68.0
335	66.0	65.0	65.5
352	66.5	67.5	67.0
357	68.0	68.0	68.0

Total Average Hematocrit	67.9

Appendix IV

Morphology at Zero Day

Total Average Morphology Index at Zero Day	100

21 Days after Thawing

Patient	Smooth Discs	Crenated Discs	Crenated Discoids & Spheroids	Crenated & Smooth Spheres	Morphology Index
204	148	45	41	18	78.28
209	112	39	16	3	86.15
217	306	130	28	10	86.84
224	141	51	17	2	87.42
248	146	44	15	9	86.11
267	135	113	64	18	73.36
340	98	33	17	5	84.08
341	124	66	22	2	84.20
343	124	39	24	16	80.07

Total Average Morphology Index	82.95

Appendix V

Micro-Drabkins Assay at Zero Day

Patient	Hemoglobin in Supernatant (mg/dL)			Average (mg/dL)
	sample 1	sample 2	sample 3	
204	51.6	50.0	50.7	50.8
209	58.3	60.6	53.0	57.3
217	67.2	73.8	73.3	71.4
224	89.9	94.6	92.9	92.5
248	101.0	100.2	101.4	100.9
249	50.6	48.6	51.1	50.1
267	74.0	76.0	74.3	74.8
334	63.5	66.0	65.0	64.8
340	79.3	79.3	78.5	79.0
341	74.2	77.6	73.1	75.0
343	82.9	77.1	75.2	78.4
344	38.2	43.0	38.8	40.0

Total Average Hemoglobin in Supernatant (mg/dL)	69.6

Appendix VI

Sodiums and Potassiums

Patient	mM Na^+/L Supernatant			mM K^+/L Supernatant		
	before freezing	0 day	14 day	before freezing	0 day	14 day
197	171	165	129	3.61	1	51
199	178	154	140	3.74	1	22
201	174	160	135	3.60	1	38
203	175	185	140	3.68	1	52
212	171	152	135	3.71	1	30
213	160	152	139	3.22	1	48
214	172	172	133	3.47	1	47
219	172	166	136	3.51	1	47
220	169	159	135	3.88	1	40
223	170	167	128	3.81	1	54
231	170	158	135	3.79	1	39
232	175	160	131	3.49	3	44
243	173	167	129	3.45	2	53
244	163	164	121	3.10	1	61
245	172	188	129	3.30	2	62
246	172	159	126	3.46	3	49
247	170	167	124	3.43	4	57
253	168	170	130	3.09	2	47
270	169	156	129	3.71	2	44
332	173	175	117	3.63	3	58

Total Averages

mM Na^+/L Supernatant			mM K^+/L Supernatant		
before freezing	0 day	14 day	before freezing	0 day	14 day
170.9	164.8	118.6	3.54	1.65	47.15

(Sodiums and Potassiums con'd)

Patient	mM Na^+/L Supernatant			mM K^+/L Supernatant		
	before freezing	0 day	21 day	before freezing	0 day	21 day
204	175	174	124	3.60	1	56
209	173	175	123	3.64	2	62
217	168	178	135	3.54	1	50
218	177	174	122	3.77	6	65
224	165	165	124	4.04	1	48
248	161	164	128	3.48	2	44
249	171	172	121	3.23	1	58
267	166	180	124	3.41	2	63
334	175	175	120	3.52	5	65
340	183	165	125	4.30	2	49
341	174	163	123	4.10	1	48
342	177	174	120	4.10	3	64
343	186	166	125	3.67	1	49
344	172	165	129	3.18	1	40
345	173	175	122	4.06	2	59
346	171	175	123	3.58	2	61
347	173	164	125	3.60	2	46
348	171	165	126	3.59	1	45
350	171	164	127	3.65	2	45
351	172	165	126	3.34	1	47

Total Averages

mM Na^+/L Supernatant			mM K^+/L Supernatant		
before freezing	0 day	21 day	before freezing	0 day	21 day
172.7	169.9	124.6	3.67	1.95	53.2

Total Compiled Averages

mM Na^+/L Supernatant		mM K^+/L Supernatant	
before freezing	0 day	before freezing	0 day
171.8	167.4	3.61	1.80

Appendix VII

Hemoglobin Assay at Zero Day

Patient	Hb/dL at Zero Day
196	24.872
268	22.7782
335	22.2814
352	22.8356
357	22.1667

Total Average Hb/dL at Zero Day	22.9298

Appendix VIII

Blood pH at Zero Day

Patient	Blood pH
196	7.033
268	7.053
335	7.021
352	6.978
357	7.053

Total Average Blood pH at Zero Days	7.028

Figure 1

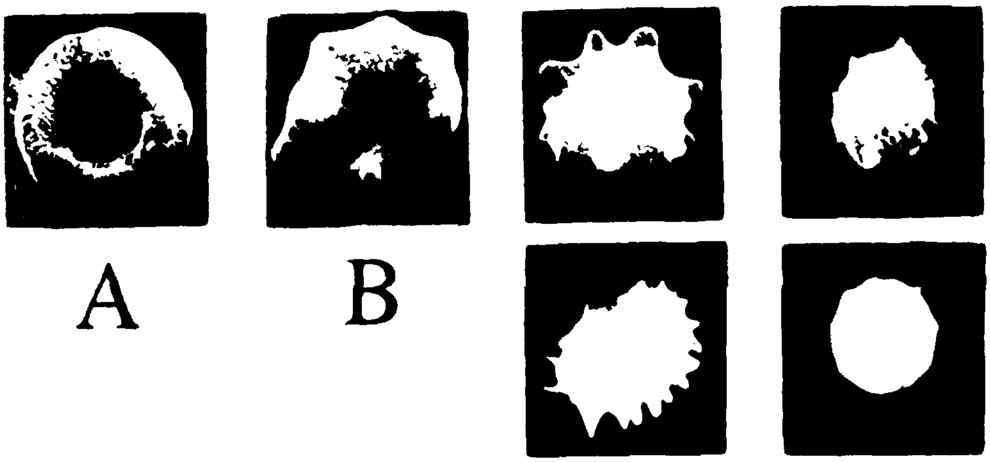
A Smooth Discs

B Crenated Discs

C Crenated Discoids and Crenated Spheroids

D Crenated Spheres and Smooth Spheres

E Total number RBCs



A B C D